Regulation of Metabolite Transport in Rat and Guinea Pig Liver Mitochondria by Long Chain Fatty Acyl Coenzyme A Esters*

(Earl Shrago, Austin Shug, Charles Elson, Terry Spenettta, and Cheryl Crosby)

From the Departments of Medicine and Nutritional Sciences, University of Wisconsin and the Veterans Administration Hospital, Madison, Wisconsin 53706

SUMMARY

Long chain fatty acyl coenzyme A esters were found to be potent inhibitors of adenine nucleotide translocation in both rat and guinea pig liver mitochondria. There was a positive correlation of inhibition with the carbon chain length and carnitine-dependent oxidation of the fatty acyl-CoA esters. Octanoyl-CoA was completely ineffective at concentrations up to 50 μM. Esters containing a greater number of carbon atoms produced significant inhibition at concentrations as low as 5 μM. Both saturated and unsaturated acyl-CoA esters were effective inhibitors, although there was some difference in effectiveness depending upon the concentration.

Palmitoyl-CoA was shown to inhibit binding competitively with ADP in both nucleotide depleted mitochondria and Lubrol membrane fragments. The mode of inhibition and sensitivity of adenine nucleotide translocase to the acyl-CoA esters appeared to be similar to that produced by atractylate. Under conditions studied, the concentration of carnitine acyltransferase enzyme(s) did not influence the inhibition produced by the acyl-CoA esters.

Phosphoenolpyruvate, known to be transported on the tricarboxylate carrier, also was found to be transported by the adenine nucleotide translocase in both rat and guinea pig liver mitochondria. In addition the tricarboxylate as well as adenine nucleotide translocator was inhibited by atractylate as well as long chain fatty acyl-CoA esters. These results suggest an important function of long chain fatty acyl-CoA esters in the regulation of mitochondrial metabolite transport.

It has been well documented, in this (1, 2) and other laboratories (3–5), that, like atractylate and bongkrekic acid, long chain fatty acyl coenzyme A esters, inhibit the translocation of adenine nucleotides across the inner mitochondrial membrane. The inhibitory effect is specific for the acyl-CoA ester and cannot be reproduced by the carnitine derivative or free fatty acid. Since long chain acyl-CoA esters can be considered natural effectors of the adenine nucleotide translocator, the significance of a physiological control of metabolite transport in the over-all modulation of cellular metabolism is readily apparent. The postulated regulation of gluconeogenesis (2), ketogenesis (6), and pyruvate oxidation (7) in liver is consistent with the coordinated changes in the concentrations of long chain fatty acyl-CoA esters, adenine, and pyridine nucleotide ratios and translocator activity (8).

The present investigation is intended to extend previous studies and to characterize the biochemical and kinetic features of certain metabolite translocators in liver mitochondria affected by long chain fatty acyl-CoA esters. Since the over-all metabolic response by guinea pig liver to administered fatty acids is considerably different than that of the rat (9, 10), mitochondria prepared from livers of both animals were compared.

MATERIALS AND METHODS

Chemicals—[U-14C]ADP, [U-14C]ATP, [1,5-14C]citrate and DL-[methyl-14C]carnitine were purchased from Amersham Searle; acyl-CoA derivatives from P.J. Biochemicals; DL-carnitine from Schwarz-Mann; atractylate, antimycin A, Lubrol WX, rotenone, and fatty acid-free albumin, type F, from Sigma; and Soluene from Packard. All other reagents were of the highest grade commercially available.

Experimental Animals—Male Sprague-Dawley rats and Hartley guinea pigs weighing approximately 200 and 350 g, respectively, were maintained on Purina laboratory chow and water ad libitum. Fasted animals were killed 48 hours after food was withheld.

Preparation of Mitochondria, Nucleotide-depleted Mitochondria, and Lubrol Membrane Fractions—Liver mitochondria were prepared by the standard procedure of Schneider (11) as previously described (2). For depletion of adenine nucleotides, mitochondria treated according to the method of Weidemann et al. (12) were suspended in a medium containing 250 mM sucrose, 10 mM phosphate, 20 mM triethanolamine-HCl (pH 7.0), 7 mM EDTA, 5 mM MgCl2, and 10 mM Tris-sulfate. The mitochondria were centrifuged at 8,000 × g for 5 min in a Sorval RC2-B centrifuge and the procedure was repeated three times using fresh media. Mitochondria were finally suspended in 250 mM sucrose, 100 mM Tris-HCl (pH 7.4), and 1.0 mM EDTA at a concentration of 20 mg of protein per ml. Preparation of a Lubrol mitochondrial membrane fraction was carried out according to the method of Winkler and Lehninger (13). Washed mitochondria at a concentration of 30 mg of protein per ml were suspended in a medium of 250 mM sucrose, 10 mM Tris-HCl (pH 7.4), and 1.0 mM EDTA. An aqueous

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solution (1.9%) of the nonionic detergent Lubrol WX was added to the suspension so that the final mixture contained 0.1 mg of Lubrol per mg of mitochondrial protein. The mixture was incubated at 0° for 30 min and centrifuged at 14,000 x g for 1 hour at 144,000 x g in a Spinco ultracentrifuge. The pellet was resuspended in the above medium at a concentration of 0.5 mg of protein per ml.

Assays Methods—[^14C]ADP translocation and binding experiments were carried out by the forward exchange reaction essentially as previously described (1, 2). A mixture containing 30 nmol Tris-HCl (pH 7.5), 75 mm KCl, 1.5 mM EDTA, and mitochondrial protein in a volume of 0.9 ml was preincubated with or without the fatty acyl-CoA esters at ice temperature for 5 min. Subsequently, 0.04 µCi of [^14C]ADP (specific activity >450 µCi per mmole) was added and after 5 min the reaction was stopped with 5.0 mm stearate. The reaction mixture then was centrifuged for 5 min at 4,000 rpm and the supernatant was removed by suction. Radioactivity in the pellet was determined in a Packard liquid scintillation spectrometer as previously described except that the precipitate was initially dissolved with 0.5 ml of Soluene.

[^14C]ATP transport was measured by the back exchange technique described by Pfaff and Klingenberg (14) and Henderson et al. (15). Approximately 200 mg of mitochondrial protein was loaded with 0.4 µCi of [^14C]ATP (specific activity >450 µCi per mmole) by incubating at ice temperature for 1 hour in a medium containing 250 mM sucrose, 4 mM Tris-HCl (pH 7.4), and 1.0 mM EDTA. The mitochondria were washed and centrifuged twice at 7000 x g and finally suspended in the above medium at a concentration of 20 mg per ml of protein. The back exchange was carried out with 3.5 mg of mitochondrial protein containing approximately 9000 dpm preincubated in a medium containing 100 mM KCl, 40 mM Tris-HCl (pH 7.4), and 1.0 mM EDTA for 5 min at ice temperature. Following addition of 0.3 mM ATP the reaction was stopped after 2 min with 5.0 mM stearate and the mitochondria were separated by centrifugation at 4000 rpm for 10 min. Solubilization and preparation of the precipitate for counting were carried out as above.

Procedures for[^14C]citrate loading and transport were essentially the same as those described by Kleineke et al. (16). Approximately 200 mg of mitochondrial protein were loaded to 7.0 ml of a mixture containing 125 mM KCl, 20 mM Tris-HCl (pH 7.4), 5.0 µg of rotenone, 14 µg of antimycin A, and 0.5 to 1.0 µCi of [1,5-^14C]citrate (specific activity, 10 to 20 mCi per mmole). After incubation at ice temperature for 10 min, 90 ml of a solution containing 250 mM sucrose, 25 mM Tris-HCl (pH 7.4), and 1.0 mM EGTA were added and the mixture was centrifuged at 10,000 x g for 10 min. The separated mitochondria were washed twice and resuspended with the above buffer. The exchange reaction was carried out in a 1.2-ml volume containing 200 mM mannitol, 25 mM sucrose, 25 mM Tris-HCl (pH 7.4), 6.0 mg of mitochondrial protein, and approximately 80,000 dpm at ice temperature for 2 min after which the mitochondria were separated by centrifugation at 18,000 x g for 10 min. Solubilization and preparation of the precipitate for counting were carried out as above. Determination of radioactivity and calculation of percentage of exchange were made as described by Kleineke et al. (16).

The carnitine acyltransferase enzyme(s) was assayed essentially as described by Hoppel and Tomec (17). The reaction mixture of 1.5 ml containing 100 mM morpholinopropane sulfonic acid (pH 7.25), 160 mM KCl, 8 mM dithioerythritol, 4 mM DL-carnitine, 2 mg per ml fatty acid-free albumin, 0.25 µCi of [methyl-[^14C]]DL-carnitine (specific activity >45 mCi per mmole), 0.4 mM long chain fatty acyl-CoA, and approximately 0.15 µg of intact or frozen-thawed mitochondria was incubated for 5 min at 37°.

The reaction was stopped with 1.5 ml 10% HCl, and after addition of 1.5 ml of butanol the mixture was centrifuged at 3,000 rpm for 5 min. The butanol supernatant was layered over 1 ml of water saturated with butanol and centrifuged again, after which 0.5 ml of the supernatant was used for measurement of radioactivity.

Protein was determined by the biuret procedure (18). Additional information is included in the legends to the figures and tables.

### RESULTS

In previous studies it was shown that only the longer chain fatty acyl-CoA esters were effective inhibitors of adenine nucleotide translocation (2). In the present experiment (Table I) the effects of a larger series of saturated and unsaturated fatty acyl-CoA esters of various chain length were compared using guinea pig as well as rat liver mitochondria. As shown, the strongest inhibition occurred upon addition of myristoyl-, palmitoyl-, and stearoyl-CoA to liver mitochondria from both animals. Octanoyl- and linoleoyl-CoA, at the concentrations tested (5 µM) were essentially noninhibitory. It may be of some significance that inhibition of the adenine nucleotide translocase by the acyl-CoA esters was more pronounced in guinea pig than in rat liver mitochondria.

A more detailed investigation of the inhibitory effects of the fatty acyl-CoA esters on adenine nucleotide translocation is shown in Figs. 1 and 2. Increasing concentrations of myristoyl-CoA esters on adenine nucleotide translocation is presented as previously described (1, 2). A mixture containing 30 mM Tris-HCl (pH 7.5), 75 mM KCl, 1.5 mM EDTA, and mitochondrial protein in a volume of 0.9 ml was preincubated with or without the fatty acyl-CoA esters at ice temperature for 5 min. Subsequently, 0.04 µCi of [^14C]ADP (specific activity >450 µCi per mmole) was added and after 5 min the reaction was stopped with 5.0 mM stearate. The reaction mixture then was centrifuged for 5 min at 4,000 rpm and the supernatant was removed by suction. Radioactivity in the pellet was determined in a Packard liquid scintillation spectrometer as previously described except that the precipitate was initially dissolved with 0.5 ml of Soluene.

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A more detailed investigation of the inhibitory effects of the fatty acyl-CoA esters on adenine nucleotide translocation is shown in Figs. 1 and 2. Increasing concentrations of myristoyl- and oleoyl-CoA up to 50 µM produced qualitatively similar progressive inhibition in both rat and guinea pig liver mitochondria. Octanoyl-CoA was completely ineffective at all concentrations tested in both animals. The lack of effectiveness of octanoyl-CoA is consistent with evidence that the inhibitory fatty acyl-CoA esters are those whose oxidation is carnitine-dependent.
served equally well as substrates for the carnitine acyltransferase which supported only a low activity, all of the acyl-CoA esters of adenine nucleotide translocation. Except with linoleoyl-CoA, esters as substrates, to determine any potential correlation of acyl-CoA specificity and carnitine acyltransferase with inhibition tested, and in all cases the inhibition was about twice as great in oleoyl- or myristoyl-CoA. Under the experimental conditions observation was confirmed (Table II). In addition, the carnitine Linoleoyl...0.33 f 0.16 1.60 f 0.13 0.10 f 0.10 1.45 f 0.15 of the more important are the ATP-dependent activation of the membrane, and the acyl transfer to the carnitine derivative on the inner membrane. During conditions conducive to increased oxidation of fatty acids, i.e. fastings, diabetes, and a high fat diet, long chain acyl-CoA synthetase activity remains essentially unchanged, whereas a variable increase in carnitine palmitoyl-acyltransferase activity has been reported (20, 21). This latter observation was confirmed (Table II). In addition, the carnitine acyltransferase enzyme(s) was assayed using a number of CoA esters as substrates, to determine any potential correlation of acyl CoA specificity and carnitine acyltransferase with inhibition of adenine nucleotide translocation. Except with linoleoyl-CoA, which supported only a low activity, all of the acyl-CoA esters served equally well as substrates for the carnitine acyltransferase enzyme(s), and in all cases there was some increase in enzyme activity in mitochondria prepared from the fasted animals. This increase during starvation may be related to mitochondrial permeability since the differences between fasted and nonfasted animals could be partially eliminated by freeze thawing the mitochondria prior to assay (results not shown). Whereas it might be postulated that low carnitine linoleoyltransferase would permit accumulation of linoleoyl-CoA and thereby account for the inhibition of adenine nucleotide translocation, additional factors must also be invoked. A number of CoA esters such as myristoyl-, palmitoyl-, and oleoyl-CoA serve as appropriate substrates for the carnitine acyltransferase which by analogy should preclude their accumulation. Octanoyl-CoA, which is neither inhibitory nor absolutely dependent upon carnitine for its oxidation, does appear to act as a substrate in the long chain carnitine acyltransferase assay. Recently, however, an octanoyl transferase independent of either the short or long chain carnitine acyltransferases has been described in rat liver mitochondria (22).

The kinetics of the inhibition of adenine nucleotide translocase in rat and guinea pig liver mitochondria by palmitoyl-CoA are shown in Figs. 3 and 4. The large pool of free nucleotides within the mitochondria have hampered previous studies determining the interaction of the nucleotides and atractylate with a carrier system (12). In the present experiments the preliminary treatment of the mitochondria with phosphate was used to partially deplete them of endogenous nucleotides. The pretreated mitochondria then were incubated with increasing concentrations of ADP, with and without palmitoyl-CoA, and the incorporation or binding of [14C]ADP was measured. For comparison an atractylate curve is also included. Due to the high endogenous adenine nucleotide content and low number of binding sites per g of protein in liver mitochondria (12), it was difficult to obtain true $K_m$ data for ADP even after preliminary treatment with phosphate. From the data, $K_m$ values of approximately 14 and $25 \mu M$ were calculated for rat and guinea pig liver mitochondria. These contrast with the lower values of 2 to 3 $\mu M$ obtained in the rat by others (12, 13) and thus represent only the upper limits of the true affinity. Certain characteristics of the acyl-CoA inhibition, however, can be ascertained. A reciprocal plot of ADP binding with substrate concentration shows that palmitoyl-CoA like atractylate (12), produces a competitive type of inhibition. A $K_i$ of 0.6 to 0.8 $\mu M$ was calculated (23) for both rat and guinea pig liver mitochondria. As part of a study on the kinetics of the adenine nucleotide translocase in rat liver mitochondria, Vignais et al. (24) derived a $K_i$ of 0.5 $\mu M$ for palmitoyl-CoA inhibition of ADP-dependent succinate oxidation.

Experiments using the Lubrol membrane preparation from rat liver mitochondria are shown in Fig. 5. Although no longer able to retain a pool of adenine nucleotides, this preparation does have the capacity to bind adenine nucleotides and is atractylate-sensitive (13). Palmitoyl-CoA produced a marked inhibition of adenine nucleotide translocase at a 10-fold lower concentration in this membrane fraction than in whole mitochondria. The results indicate a $K_m$ value for ADP of less than 0.5 $\mu M$ and a $K_i$ value for palmitoyl-CoA of 0.3 $\mu M$ which is more compatible with expected values. It should be noted that retention of the acyl-CoA as well as atractylate sensitivity by the submitochondrial particle is additional evidence for a common action of the inhibitors at the same site on the membrane.

We previously have reported a close functional relationship between the adenine nucleotide and tricarboxylate carrier systems in rat liver mitochondria (25). The results showing an inhibition of phosphoenolpyruvate as well as citrate transport by long chain

TABLE II

<table>
<thead>
<tr>
<th>Fatty acyl-CoA ester</th>
<th>Rat</th>
<th>Guinea pig</th>
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<tr>
<td></td>
<td>nmoles/min/mg protein</td>
<td></td>
<td>nmoles/min/mg protein</td>
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<tr>
<td>Octanoyl...</td>
<td>3.91 ± 1.24</td>
<td>4.82 ± 0.87</td>
<td>3.12 ± 0.74</td>
<td>6.21 ± 0.61</td>
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<td>Lauryl...</td>
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<td>9.40 ± 0.12</td>
<td>7.47 ± 0.77</td>
<td>5.60 ± 0.73</td>
</tr>
<tr>
<td>Myristoyl...</td>
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<td>13.88 ± 0.74</td>
<td>7.73 ± 0.67</td>
<td>6.26 ± 0.94</td>
</tr>
<tr>
<td>Palmitoyl...</td>
<td>6.29 ± 0.54</td>
<td>10.50 ± 0.81</td>
<td>7.84 ± 0.38</td>
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<tr>
<td>Linoleoyl...</td>
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<td>1.60 ± 0.13</td>
<td>0.10 ± 0.10</td>
<td>1.45 ± 0.15</td>
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</table>

(1, 2, 19). Linoleoyl-CoA caused inhibition at concentrations greater than 6 $\mu M$, but never approached the effectiveness of oleoyl- or myristoyl-CoA. Under the experimental conditions shown myristoyl-CoA was the most inhibitory of the CoA esters tested, and in all cases the inhibition was about twice as great in guinea pig as in rat liver mitochondria.

The intramitochondrial concentration of long chain acyl-CoA esters is dependent upon a number of enzymatic reactions; two of the more important are the ATP-dependent activation of the fatty acid to the acyl-CoA ester on the outer mitochondrial membrane, and the acyl transfer to the carnitine derivative on the inner membrane. During conditions conducive to increased oxidation of fatty acids, i.e. fastings, diabetes, and a high fat diet, long chain acyl-CoA synthetase activity remains essentially unchanged, whereas a variable increase in carnitine palmitoyl-acyltransferase activity has been reported (20, 21). This latter observation was confirmed (Table II). In addition, the carnitine acyltransferase enzyme(s) was assayed using a number of CoA esters as substrates, to determine any potential correlation of acyl CoA specificity and carnitine acyltransferase with inhibition of adenine nucleotide translocation. Except with linoleoyl-CoA, which supported only a low activity, all of the acyl-CoA esters served equally well as substrates for the carnitine acyltransferase

FIG. 2. Effect of increasing concentrations of various long chain fatty acyl-CoA esters on the uptake of $[^{14}C]ADP$ by guinea pig liver mitochondria. The experiments were carried out as described under Fig 1.
fatty acyl-CoA esters confirmed and extended a recent report of Halperin and co-workers (26). The effects of oleoyl-CoA, atractylate, and 1,2,3-benzene tricarboxylate on the [14C]ATP and [14C]citrate exchange reactions in rat and guinea pig liver mitochondria are compared in Table III. In all respects the qualitative effects of the inhibitors in rat and guinea pig liver mitochondria are similar.

In mitochondria from both animals, phosphoenolpyruvate could be transported with ATP. The translocation of [14C]citrate with phosphoenolpyruvate on the tricarboxylate carrier system in included. $1/V_i$ represents 1/ADP bound (nanomoles per min per mg of protein). Control, ■——■; palmitoyl-CoA, 1.0 nmole, △——△; palmitoyl-CoA, 2.0 nmole, ▲——▲; palmitoyl-CoA, 5.0 nmole, ○——○; atractylate, 5.0 nmole, ●——●.

Fig. 4 (right). Double reciprocal plot of palmitoyl-CoA inhibition of the concentration-dependent rate of ADP uptake by nucleotide-depleted guinea pig liver mitochondria. Experiments were carried out and data expressed as described in Fig. 3. Control, △——△; palmitoyl-CoA, 2.0 nmole, ▲——▲; palmitoyl-CoA, 5.0 nmole, ○——○; atractylate, 5.0 nmole, ●——●.

<table>
<thead>
<tr>
<th>TABLE III</th>
</tr>
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<tr>
<td>Transport of [14C]ATP and [14C]citrate with various anions and inhibition with 1,2,3-benzene tricarboxylate, atractylate, and oleoyl-CoA</td>
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</tbody>
</table>

Results expressed as per cent exchange as described under "Materials and Methods" are average of duplicate incubation from a representative experiment. An absence of exchange activity is expressed by a negative sign (-). Negative values indicate that in the presence of that anion the counts in the supernatant were lower than when incubated in buffer only. The concentration of externally added citrate was 10 mM; ATP and ADP, 0.1 mM; and phosphoenolpyruvate, 5.0 mM for rat and 1.0 mM for guinea pig. The concentration of inhibitors were 5 mM oleoyl-CoA, 0.5 mM atractylate, and 50 mM 1,2,3-benzene tricarboxylate (1,2,3-BTCA).

<table>
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<tbody>
<tr>
<td></td>
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<td>1,2,3-BTCA</td>
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<td>(−)</td>
</tr>
<tr>
<td>Citrate</td>
<td>Atractylate</td>
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<td>(−)</td>
</tr>
<tr>
<td>Citrate</td>
<td>Oleoyl-CoA</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>Phosphoenolpyruvate</td>
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<td>Atractylate</td>
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Fig. 3 (left). Double reciprocal plot of palmitoyl-CoA inhibition of the concentration-dependent rate of ADP uptake by nucleotide-depleted rat liver mitochondria. Rat liver mitochondria treated with phosphate buffer to deplete them of endogenous adenine nucleotides were incubated with varying concentrations of [14C]ADP with and without palmitoyl-CoA and assayed for adenine nucleotide translocase activity as described under "Materials and Methods." Double reciprocal plots are shown for the effect of palmitoyl-CoA on the concentration-dependence of [14C]ADP uptake. A comparison with a curve for atractylate is also included. $1/V_i$ represents 1/ADP bound (nanomoles per min per mg of protein). Control, ■——■; palmitoyl-CoA, 1.0 nmole, △——△; palmitoyl-CoA, 2.0 nmole, ▲——▲; palmitoyl-CoA, 5.0 nmole, ○——○; atractylate, 5.0 nmole, ●——●.

Fig. 5. Double reciprocal plot of palmitoyl-CoA inhibition of the concentration-dependent rate of [14C]ADP uptake by the Lubrol membrane fraction of rat liver mitochondria. The reaction was carried out as described in Fig. 3 using the Lubrol membrane fraction of rat liver mitochondria prepared as described under "Materials and Methods." Double reciprocal plots are shown for the effect of palmitoyl-CoA on the concentration-dependent ADP binding. A comparison with a curve for atractylate is also included. Control, △——△; palmitoyl-CoA, 0.1 nmole, ▲——▲; palmitoyl-CoA, 0.5 nmole, ○——○; atractylate, 5 nmole, ●——●.
animals. At all concentrations tested, the various acyl-CoA translocase in guinea pig liver. The mechanism by which inhibition of adenine nucleotide translocation could exert a negative sized in large quantities, to the cytosol for subsequent conversion
zyme Institute, University of Wisconsin, Madison, Wisconsin.

Discussion

It is well recognized that the adenine nucleotide translocase system plays a central role in cellular metabolism. The reversible inhibition of adenine nucleotide translocation by long chain fatty acyl-CoA esters represents the physiological mechanism for functionally linking the metabolism of the mitochondrial and cytosolic compartments. The changes in the adenine nucleotide ratios and oxidation-reduction states in the mitochondria and cytosol compartments which result from the inhibited translocase can in turn influence rates of gluconeogenesis, ketogenesis, and energy-linked respiration (1-8, 27, 28).

Inhibition of adenine nucleotide translocase by atractylate (29, 30) and bongkrekic acid (31, 32) has been used experimentally to characterize this carrier system. The kinetic studies with palmitoyl-CoA indicate that like atractylate, acyl-CoA esters are competitive with adenine nucleotides for binding to the translocase carrier. It is likely that the similarity of the adenine group of the CoA moiety to the free adenine nucleotide accounts for the inhibition. Since neither free CoA nor short chain acyl-CoA esters are inhibitory (2), acyl chain length and steric configuration may be important factors. Of interest were the results obtained using the vesicular membrane preparation from Lubrol-treated mitochondria. As described by Winkler and Lehninger (13), this particle, which results from a loss of 70% of the mitochondrial protein into the soluble form still retains a capacity to bind nucleotides in an atactic translocase sensitive manner. This membrane preparation also proved extremely susceptible to inhibition by palmitoyl-CoA, thereby providing additional evidence that the acyl-CoA esters act as natural effectors in the same manner as atractylate.

In contrast to their action as positive effectors in rat liver, long chain fatty acids appear to exert a negative effect on gluconeogenesis in guinea pig liver (9, 10). For this reason, the effects of long chain acyl-CoA esters on adenine nucleotide translocation were compared in liver mitochondria prepared from both these animals. At all concentrations tested, the various acyl-CoA esters produced a more marked inhibition of adenine nucleotide translocase in guinea pig liver. The mechanism by which inhibition of adenine nucleotide translocation could exert a negative influence on gluconeogenesis in the guinea pig is not completely clear. One possibility is an inhibition of transport of phosphoenolpyruvate from guinea pig mitochondria, where it is synthesized in large quantities, to the cytosol for subsequent conversion to glucose.

In addition to those control mechanisms alluded to in this communication, a wide variety of physiological and pathophysiological conditions can be directly or indirectly affected by changes in the mitochondrial transport systems. The deleterious effect of fatty acids on the ischemic myocardium may be due to their impaired oxidation, resulting in increased levels of long chain acyl-CoA esters and a severely inhibited translocase (33, 34). Altered mitochondrial functions in ethanol-induced fatty livers have been associated with elevated levels of long chain acyl-CoA esters and inhibited adenine nucleotide translocation (35). Whereas the calorigenic action of thyroid hormone has been postulated to be a direct effect on the adenine nucleotide translocator (36), the possibility of an indirect effect secondary to lowering of liver long chain fatty acyl-CoA esters was not ruled out. The metabolic role of citrate in the critical regulation of acetyl-CoA carboxylase and phosphofructokinase as well as a substrate for fatty acid synthesis may in turn be related to the effects of long chain acyl-CoA esters on the mitochondrial triacyl-CoA carrier system (25, 26). Of the most important regulatory sites in cell metabolism is the pyruvate dehydrogenase complex. An increased mitochondrial ATP:ADP ratio secondary to an inhibited adenine nucleotide translocase would favor phosphorylation and inactivation of pyruvate dehydrogenase (31). Stimulation of pyruvate dehydrogenase to its active form by insulin (38) may also occur via an equivalent modification through changes in adenine nucleotide levels. As shown by Stucki et al. (7), pyruvate carboxylase activity is critically dependent upon the mitochondrial ATP:ADP ratio. Recently phosphoenolpyruvate was found to stimulate the egress of calcium from both heart and liver mitochondria and this effect could be prevented by ATP or atractylate (39). The results imply a participation of adenine nucleotide translocase which may be transporting phosphoenolpyruvate. Further studies are, however, necessary to determine definitive physiological regulatory mechanisms of the mitochondrial carrier system on cell metabolism.

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References


1 This possibility was suggested by Professor H. A. Lardy, Enzyme Institute, University of Wisconsin, Madison, Wisconsin.
Regulation of Metabolite Transport in Rat and Guinea Pig Liver Mitochondria by Long Chain Fatty Acyl Coenzyme A Esters
Earl Shrago, Austin Shug, Charles Elson, Terry Spennetta and Cheryl Crosby


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