

FATTY ACID INHIBITION OF WATER ABSORPTION AND ENERGY PRODUCTION IN THE HAMSTER JEJUNUM

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1. Introduction

A variety of fatty acids, when present within the lumen of the bowel, inhibit intestinal absorption of sodium and water. This effect accounts for the catharsis induced by castor oil (the active component of which is ricinoleic acid) and probably the diarrhea that usually occurs in fat malabsorption [1–3]. The mechanism of this effect, however, is unknown. In other tissues acyl CoA esters of long chain fatty acids are known to be potent inhibitors of adenine nucleotide translocase (ANT) [4,5,6,7], the carrier responsible for mitochondrial ADP and ATP transport [8]. We considered the possibility that the fatty acid effect on intestinal transport is mediated by inhibition of ANT. This study demonstrates that certain hydroxylated and non-hydroxylated octadecenoates inhibit intestinal water transport and ANT activity. We suggest that the acyl CoA esters of these fatty acids, by inhibiting ANT,

alter intestinal mucosal absorption by limiting the ATP available for intestinal sodium and water transport.

2. Materials and methods

Non-fasted male Golden Syrian hamsters supplied by Sprague-Dawley, Madison, Wisconsin and weighing 90 to 120 g were used. Water transport was assessed using everted gut sacs prepared from 12 cm segments of jejunum distal to the ligament of Treitz as previously described [9]. Mucosal and serosal buffer solution was gassed with 95% O₂–5% CO₂ and contained (mM) Na⁺ 150, Cl⁻ 130, K⁺ 5, HCO₃⁻ 25, and glucose 2 g/liter and polyethylene glycol 4000 (PEG) 5 g/liter. The serosal compartment contained 1.0 ml of buffer to which was added 0.02 μCi of [¹⁴C] PEG 4000 (New England Nuclear Corp., Boston, Massachusetts) as an indicator of water movement [10]. Sacs were incubated

for 30 min in 12 ml of continuously gassed buffer at 37°C. One hundred μ l aliquots of serosal solution were taken in duplicate at the beginning and at the end of incubation and counted in 10 ml of Insta-Gel scintillation fluid (Packard Instrument, Downers Grove, Illinois), using a Packard 2002 liquid scintillation spectrometer. Total recovery of [14 C] PEG 4000 was $98.5 \pm 1.2\%$ ($n = 6$). Standard formulae [9] were used to determine net water absorption. The fatty acid to be tested was added to the mucosal fluid at a concentration of 4 mM. ANT activity was measured, as previously described [11], directly in the homogenate of mucosal scrapings of sacs incubated without [14 C] PEG. The mucosa from 3 sacs was pooled and homogenized in about 10 ml of ice-cold EGTA buffer (250 mM sucrose, 1 mM EGTA, 4 mM Tris chloride – pH 7.4), and then brought to a final volume of 20 ml with buffer. ANT activity was then determined directly in the homogenate by addition of aliquots to the following medium: 110 KCl, 30 mM Tris, 1 mM EDTA, and 0.48 mM [14 C] ADP (240 000 dpm). The reaction was initiated by addition of the homogenate, run for 5 min on ice, and terminated by the addition of atracylate (an ANT-specific inhibitor) [12]. Seven ml of EGTA buffer were added, and the tubes centrifuged at 20 300 g for 10 min. The supernatant was decanted, the pellet washed once with EGTA buffer, and the pellet (containing mitochondria and nuclei) solubilized with 0.2 ml of Soluene and counted in Bray's counting fluid. A duplicate tube containing 0.5 mM atracylate was always run as a blank and results are expressed as atracylate-sensitive ANT activity. The DPM found in the mito-

chondrial fraction (nuclei do not incorporate significant counts under the conditions of the assay), were a measure of ANT activity [4].

In addition, purified mitochondria were prepared for respiratory studies. Jejunal scrapings from 18 fasted hamsters were pooled and homogenized in 20 ml of ice-cold EGTA buffer. This mixture was centrifuged for 10 min at 600 g and the supernatant then centrifuged for 10 min at 5000 g . The mitochondrial pellet was washed once in EGTA media containing 0.05% BSA (bovine serum albumin), re-centrifuged, and then brought up to about 2 ml in the buffer so that 100 μ l of the mitochondrial suspension contained about 0.05 mg protein.

The following fatty acids, as sodium salts, were studied: ricinoleate, elaidate (Nu-Chek Preparations, Elysian, Minnesota), ricinelaidate, and oleate (Sigma Chemical Co., St. Louis, Missouri). Oleoyl-CoA was obtained from P-L Labs (Milwaukee, Wisconsin) and DL-carnitine came from Sigma. All fatty acids were pure as checked by thin-layer chromatography. Polygram Sil G/UV plastic plates (Brinkman Instruments, Inc.) were used with a solvent system of light petroleum, anhydrous diethyl ether, and acetic acid (70:30:3). Data analysis consisted of a standard unpaired t-test, comparing control with drug-treated tissue [13].

3. Results and discussion

Ricinoleate, ricinelaidate, and oleate inhibit ANT activity. Table 1 demonstrates this inhibition and shows

Table 1
Inhibition of net water absorption and mitochondrial adenine nucleotide translocase in the hamster jejunum

Fatty acid (4.0 mM)	Percent inhibition of water absorption (\pm S.E.M.)	Percent inhibition of adenine nucleotide translocase
Ricinoleate (<i>cis</i>)	80.3 ± 1.6 (6) *	28.5 ± 1.5 (4)
Ricinelaidate (<i>trans</i>)	69.7 ± 7.7 (7) *	18.0 (2)
Oleate (<i>cis</i>)	21.9 ± 5.5 (6) *	34.8 ± 3.1 (4)
Elaidate (<i>trans</i>)	None (6) **	2.5 (2)

Numbers in parantheses are the number of experiments performed.

* $P < 0.05$ compared to respective control.

** Net water absorption in μ l/gm dry tissue was: control 238.2 ± 10.2 elaidate 245.9 ± 27.3 .

the relationship between the effects on ANT and water absorption. Elaidate, which does not inhibit rat liver ANT [14], did not inhibit ANT or water transport, indicating that the inhibition is not the result of a non-specific effect of fatty acids or their CoA esters. It should be emphasized that ricinoleate and ricinelaide inhibited water transport much more than did oleate, even though ANT inhibition was slightly more with oleate. This strongly suggests that the hydroxy fatty acids inhibit water transport by another mechanism, as well as by inhibiting ANT.

In an effort to determine more precisely the relationship between ANT and water transport, the ANT-specific inhibitor atractylate was added to the mucosal compartment of gut sacs at a concentration of 19 mM. Appropriate adjustments were made for the slight change in osmolality. It was observed that water transport was only inhibited 40%; while, ANT inhibition was about 90%. In these experiments the mucosa was thoroughly washed in an effort to remove adhering atractylate which could cause artifactually high ANT inhibition; however, less than 10% of ANT inhibition could have been caused by contaminating atractylate. The lack of

correlation between water transport and ANT inhibition by atractylate therefore suggests, as others have found [15], that in jejunal mucosa, glycolysis supplies a large part of the energy needed for the absorptive process.

In preliminary experiments we have demonstrated that mucosal levels of acyl CoA esters increase from a control value of 10 nmol/g frozen weight to a value of 30 nmol/g frozen weight during incubation with 4 mM ricinoleate. This finding is in keeping with previous studies [7,16] which have established that increases in the intracellular level of acyl CoA esters results in ANT inhibition. To determine more directly the effects of the acyl CoA esters, mitochondria were prepared for respiratory studies. They were in good functional state; respiratory control, state 3 (ADP present/state 4 (ADP absent)), was often greater than 15, and the rate of O₂ consumption in the coupled state equalled that in the uncoupled state. ADP/O ratios were about 3.5 for glutamate plus malate, and 2.5 for succinate. Trace A in fig.1 demonstrates the high respiratory activity of these mitochondria, comparable, in fact, to those from cardiac muscle [17]. Trace B shows inhibition of respiration by oleoyl CoA; this inhibition

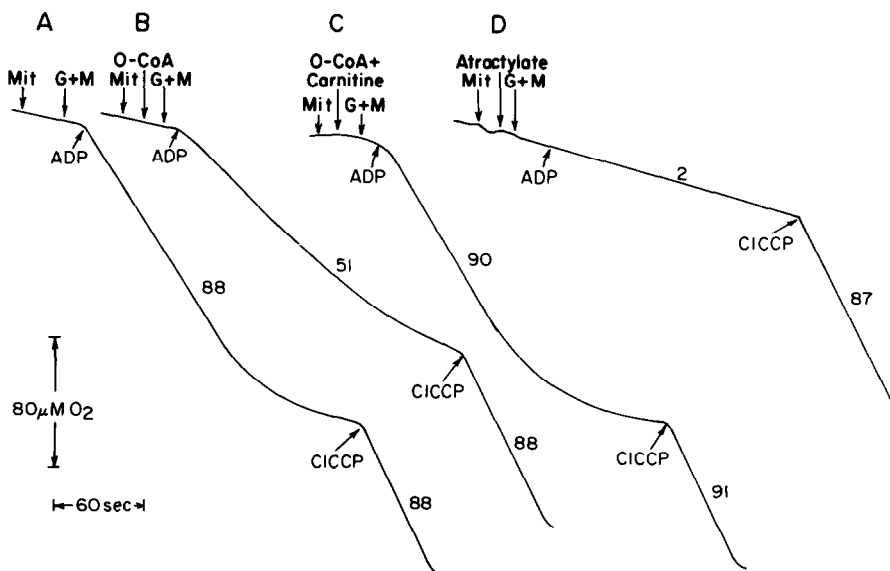


Fig.1. The inhibition of state 3 respiration in mucosal mitochondria. The standard 1.0 ml incubation mixture run at 22°C contained 230 mM mannitol, 70 mM sucrose, 20 mM Tris-HCl, pH 7.4, 5 mM K₂HPO₄, 20 μM EDTA, and 2.0 mg mitochondrial protein. Additions were: 7.0 mM Na glutamate + 7.0 mM Na malate, (G+M); 0.4 mM ADP, 33 μM carbonyl cyanide *m*-chlorophenylhydrazone (CICCP) and 5.0 mM D,L-carnitine. 2 μM of oleoyl CoA (O-CoA) and 5 μM of atractylate were added at points indicated. The numbers on the recorder traces give the rates of oxygen consumption in μmol/liter/min.

is similar to that seen in mitochondria from other tissues by long chain acyl CoA esters [18]. The inhibition in the coupled state was reversed by the addition of uncoupler (CICCP) indicating it was the consequence of inability of ADP to penetrate the inner mitochondrial membrane. Trace C indicates complete metabolism of oleoyl CoA to the non-inhibiting acylcarnitine derivative by carnitine acyltransferase [7,19]. Trace D demonstrated inhibition by the specific ANT inhibitor, atractylate. As with the oleoyl CoA effect, the inhibition was completely reversed by addition of uncoupler.

This study confirms observations in other tissues which have indicated that addition of long chain acyl CoA esters inhibits ANT activity [4]. A positive correlation between tissue concentrations of long chain acyl CoA esters and ANT inhibition already has been demonstrated in liver [18] and cardiac muscle [16]. Reversal by carnitine (fig.1) confirms previous observations in other tissues that only the CoA ester is inhibitory [4,5,7,19].

In summary, these results indicate that in intestine as with other organs, ANT, by controlling the mitochondrial ATP/(ADP + P_i) ratio can influence the regulation of energy-linked cellular metabolism [6,20,21,22]. Intracellular accumulation of acyl CoA esters during incubation of gut sacs with fatty acids has been shown to inhibit ANT activity, thus causing a decrease in supply of mitochondrial ATP available for water transport. However, it is likely [15] that mucosal mitochondria supply only a portion of total ATP requirements for cellular water transport in jejunum. These results, in part, account for the effects of fatty acids on water absorption.

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