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Effect of Growth Hormone on Fatty Acid Oxidation: Growth Hormone Increases the Activity of 2,4-Dienoyl-CoA Reductase in Mitochondria[1]

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The effect of growth hormone on the β-oxidation of saturated and unsaturated fatty acids was studied with mitochondria isolated from control rats, hypophysectomized rats, and hypophysectomized rats treated with growth hormone. Rates of respiration supported by polyunsaturated fatty acylcarnitines, in contrast to rates observed with palmitoylcarnitine or oleoylcarnitine, were slightly lower in hypophysectomized rats than in normal rats, but were higher in hypophysectomized rats treated with growth hormone. The effects were most pronounced with docosahexaenoylcarnitine, the substrate with the highest degree of unsaturation. Since uncoupling of mitochondria with 2,4-dinitrophenol resulted in lower rates of docosahexaenoylcarnitine-supported respiration, while substitution of ATP for ADP yielded higher rates, it appears that energy is required for the effective oxidation of polyunsaturated fatty acids. Growth hormone treatment of hypophysectomized rats caused a threefold increase in the activity of 2,4-dienoyl-CoA reductase or 4-enoyl-CoA reductase (EC 1.3.1.34) in mitochondria, but not in peroxisomes. The activities of other β-oxidation enzymes remained virtually unchanged. Rates of acetoacetate formation from linolenoylcarnitine, but not from palmitoylcarnitine, were stimulated by glutamate in mitochondria from hypophysectomized rats and hypophysectomized rats treated with growth hormone. All data together lead to the conclusion that the mitochondrial oxidation of highly polyunsaturated fatty acids is limited by the availability of NADPH and the activity of 2,4-dienoyl-CoA reductase which is induced by growth hormone treatment.

Growth hormone affects the structure and function of liver mitochondria in rat (1-6). Removal of the hypophysis results in a deficiency of growth hormone (somatotropin), prolactin, and tropic hormones like adrenocorticotropic hormone, thyroid stimulating hormone, and gonadotropins. Treatment of hypophysectomized rats with growth hormone causes increases in body weight and skeletal growth and thus provides a model for studying the biochemical actions of growth hormone related to its growth promoting property in the absence of thyroxine, cortisone, testosterone, and estrogen (4-6).

Growth hormone stimulates mitochondrial protein synthesis (1) and causes changes in the composition of mitochondrial phospholipids (4). More specifically, growth hormone treatment of hypophysectomized rats leads to the incorporation of more polyunsaturated fatty acids, like docosahexaenoic acid, into mitochondrial phospholipids (4). This change in the composition of mitochondrial phospholipids results in an altered membrane fluidity which may be the cause for activity changes of some membrane-bound en-

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zymes. For example growth hormone treatment of hypophysectomized rats causes increases in the activities of NADH dehydrogenase and the energy-dependent transhydrogenase (3).

The observed structural and functional changes of liver mitochondria in response to growth hormone treatment of hypophysectomized rats prompted us to study the effect of this hormone on the mitochondrial oxidation of fatty acids. We were especially interested in the oxidation of polyunsaturated fatty acids because their degradation requires NADPH for the reductive removal of double bonds extending from even-numbered carbon atoms (7–10). This reaction is catalyzed by 2,4-dienoyl-CoA reductase or 4-enoyl-CoA reductase (EC 1.3.1.34). In this report we provide evidence for growth hormone-dependent increases in the mitochondrial activity of 2,4-dienoyl-CoA reductase and the mitochondrial capacity to oxidize polyunsaturated fatty acids.

EXPERIMENTAL PROCEDURES

Materials. Palmitic acid, oleic acid, linoleic acid, linolenic acid, arachidonic acid, and docosahexaenoic acid were purchased from Nu Check Prep. 4-Pentenoic acid and 3-hydroxyacyl-CoA dehydrogenase were purchased from Fluka, A. G., Switzerland, and Boehringer-Mannheim, FRG, respectively. Glutamic acid, malonic acid, acetoacetic acid, coenzyme A, butyryl-CoA, palmitoyl-CoA, rotenone, ADP, ATP, sodium succinate, palmitoylcarnitine, L-carnitine, and other chemicals were supplied by Sigma. Unsaturated acylcarnitines were prepared by the method of Cervenka and Osmundsen (11). According to their procedure fatty acids are reacted with a 10% molar excess of *N,N*-carbonyldiimidazole to yield *N*-acylimidazoles which are reacted with carnitine perchlorate to form the acylcarnitines. Fatty acylcarnitines were purified by high-performance liquid chromatography on reverse-phase Sep-Pak cartridges (11), and stored as methanolic solutions in the presence of the antioxidant butylated hydroxytoluene. Crotonyl-CoA and acetoacetyl-CoA were prepared according to standard procedures described by Weeks and Wakil (12) and Seubert (13), respectively. *2-trans,4-trans*-Decadienoyl-CoA was synthesized by the mixed anhydride procedure as described by Cuebas and Schulz (8).

Animals and hormone treatments. Male Sprague-Dawley rats were maintained on Purina Laboratory Chow *ad libitum.* Control rats weighed 200–250 g, hypophysectomized rats 100–150 g, and growth hormone-treated rats 200–220 g, respectively. Hypophysectomized rats were provided with 5% dextrose solution instead of water. Bovine growth hormone (NIH, BG-17), 0.1 IU/100 g body wt, was injected subcutaneously daily for 7 days (2).

Isolation of mitochondria and peroxisomes. Rats were sacrificed and livers were removed and washed with ice-cold isolation buffer consisting of 0.25 M sucrose, 1 mM EDTA, 30 mM Tris-HCl (pH 7.4), and 0.5% bovine serum albumin. Mitochondria were isolated and washed as previously described (2). Mitochondria and peroxisomes were separated by density gradient centrifugation as described in principle by Osumi and Hashimoto (14). Light mitochondria (40 mg) were placed on top of a sucrose gradient composed of 10 ml of 55% sucrose, 10 ml of 44.75% sucrose, and 10 ml of 33% sucrose. All sucrose solutions contained additionally the components of the isolation buffer. Centrifugation in a vertical rotor (Type SS 90) was performed for 75 min at 34,600*g*. Fractions were collected from the bottom of the centrifuge tubes and assayed for marker enzymes and various β-oxidation enzymes as described by Dommes *et al.* (15). Peroxisomal fractions were essentially free of mitochondria, but mitochondrial fractions contained some peroxisomes as judged by the distribution of catalase.

Measurements of mitochondrial respiration. Rates of acylcarnitine-supported respiration were measured polarographically with a Clark oxygen electrode at 30°C as previously described (3, 4). Measurements were performed in the presence of 5 mM malonate and 2 mM ADP, or 2 mM ATP, or 50 μM 2,4-dinitrophenol. When rates of respiration were measured in the presence of 4-pentenoate, mitochondria (2 mg/ml) were preincubated with 0.1 mM 4-pentenoate in the presence of 2 mM ADP and 5 mM malonate at 25°C for the indicated periods of time. Respiration was initiated by the addition of linolenoylcarnitine. When the preincubation time was zero, 4-pentenoate and linolenoylcarnitine were added simultaneously.

Measurements of β-oxidation. Rates of β-oxidation were determined by measuring spectrophotometrically the acylcarnitine-dependent reduction of ferricyanide (16). The assay mixture contained in addition to the standard components (16) liver mitochondria (2–4 mg/ml), rotenone (2 μg/ml), 40 μM acylcarnitines, and 10 mM oxaloacetate.

Measurements of acetoacetate formation. Liver mitochondria (2 mg/ml) from rats fasted for 24 h were incubated in the presence of 3.3 mM malonate and 30 μM acylcarnitines as previously described (3, 4). The reaction was started by the addition of 2 mM ADP and allowed to proceed for 5 min at which time the reaction was terminated by acidification with $HClO_4$. The concentration of acetoacetate was determined enzymatically with 3-hydroxybutyrate dehydrogenase (18).

Enzyme assays. 2,4-Dienoyl-CoA reductase was assayed by recording the decrease in absorbance due to

the oxidation of NADPH (7). An assay mixture contained 48 mM KP_i (7.4), 0.1 mM NADPH, 80 μM 2-*trans*,4-*trans*-decadienoyl-CoA, and 2,4-dienoyl-CoA reductase (8 mU/ml). Catalase (EC 1.11.1.6) was assayed spectrophotometrically as described by Aebi (19). Butyryl- and acyl-CoA dehydrogenases (EC 1.3.99.2 and EC 1.3.99.3) were assayed by measuring at 600 nm the reduction of 2,6-dichlorophenolindophenol with phenazine methosulfate as a primary electron acceptor (20). Butyryl-CoA and palmitoyl-CoA served as substrates of butyryl-CoA dehydrogenase and acyl-CoA dehydrogenase, respectively. 3-Hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) was assayed in the backward direction by measuring the oxidation of NADH at 340 nm with acetoacetyl-CoA as a substrate (21). The activities of thiolases (EC 2.3.1.9 and EC 2.3.1.16) were determined spectrophotometrically at 303 nm by recording the disappearance of the Mg^{2+}-enolate complex of acetoacetyl-CoA (22).

RESULTS

The effects of hypophysectomy and hypophysectomy followed by treatment with growth hormone (referred to as growth hormone treatment) on hepatic fatty acid oxidation in rat were evaluated by measuring rates of acylcarnitine-supported respiration with isolated rat liver mitochondria (see Table I). Measurements with mitochondria from control animals yielded much lower rates of respiration with arachidonoylcarnitine and docosahexaenoylcarnitine as compared to rates obtained with the carnitine derivatives of palmitic acid, oleic acid, linoleic acid, and linolenic acid. All rates are very similar to those reported by Osmundsen *et al.* (17). The low oxidation rates observed with arachidonoylcarnitine and docosahexaenoylcarnitine may be a consequence of their longer chain lengths or their higher degrees of unsaturation. With mitochondria from control animals, all rates of respiration were slightly increased when mitochondria were uncoupled with 2,4-dinitrophenol, whereas the substitution of ATP for ADP had no significant effect on rates of respiration (data not shown). Hypophysectomy changed this picture little except that the rate with palmitoylcarnitine was increased, whereas those obtained with arachidonoylcarnitine and docosahexaenoylcarnitine were further reduced (see Table I). However, hypophysectomy followed by treatment with growth hormone altered the picture profoundly. In the presence of ADP, rates of respiration with all polyunsaturated fatty acylcarnitines were increased, whereas rates with palmitoylcarnitine and oleoylcarnitine were hardly affected (see Table I). The rate of respiration supported by docosahexaenoylcarnitine was increased more than threefold compared to rates observed with mitochondria

TABLE I

EFFECTS OF HYPOPHYSECTOMY AND GROWTH HORMONE TREATMENT ON MITOCHONDRIAL RESPIRATION SUPPORTED BY ACYLCARNITINES IN THE PRESENCE OF MALONATE[a]

	Rates of respiration (ng atom O/min/mg protein)[b]				
			Growth hormone		
Acylcarnitine	Control +ADP	Hypophysectomized +ADP	+ADP	+DNP	+ATP
Palmitoylcarnitine	28 ± 8	43 ± 7	25 ± 6	48 ± 8	24 ± 6
Oleoylcarnitine	36 ± 5	39 ± 6	44 ± 7	60 ± 5	33 ± 5
Linoleoylcarnitine	29 ± 8	27 ± 10	52 ± 6	54 ± 4	47 ± 6
Linolenoylcarnitine	32 ± 4	28 ± 5	49 ± 5	48 ± 5	33 ± 4
Arachidonoylcarnitine	9 ± 3	5 ± 2	28 ± 2	22 ± 4	40 ± 6
Docosahexaenoylcarnitine	7 ± 3	3 ± 2	24 ± 8	12 ± 3	40 ± 6

[a] Rates of respiration were measured polarographically at 30°C with liver mitochondria (2 mg/ml) in the presence of 40 μM acylcarnitines, 5 mM malonate, 2 mM ADP, or 2 mM ATP, or 50 μM 2,4-dinitrophenol (DNP).

[b] Values are means ± SD. Samples from at least five animals were assayed in triplicate each.

from control animals and eightfold when compared to hypophysectomized rats. Uncoupling with 2,4-dinitrophenol stimulated respiration supported by palmitoylcarnitine and oleoylcarnitine, but inhibited respiration sustained by arachidonoylcarnitine and docosahexaenoylcarnitine (see Table I). When ATP was used instead of ADP, rates with arachidonoylcarnitine and docosahexaenoylcarnitine, in contrast to all other substrates, were substantially increased (see Table I). All observations together prompt the conclusions that (a) an inverse relationship exists between the degree of unsaturation of fatty acids and their rate of mitochondrial oxidation and (b) hypophysectomy followed by growth hormone treatment results in higher oxidation rates of polyunsaturated fatty acids without affecting the oxidation of saturated and monounsaturated fatty acids. Although the mitochondrial preparations used in these measurements were not free of peroxisomes, the reported rates of respiration reflect only the mitochondrial oxidation of fatty acids, because fatty acid oxidation in isolated peroxisomes in contrast to β-oxidation in mitochondria requires the addition of NAD^+ to the incubation medium (23). This assumption is supported by the almost complete inhibition of respiration observed in the presence of cyanide (data not shown).

The effects of hypophysectomy and growth hormone treatment were also evaluated by measuring acylcarnitine-dependent rates of ferricyanide reduction in rotenone-poisoned rat liver mitochondria. With mitochondria from hypophysectomized rats all substrates yielded virtually identical rates in the absence of oxaloacetate (see Table II). In the presence of oxaloacetate, which facilitates the reoxidation of NADH, rates were increased up to 3.5-fold, except for the unchanged rate obtained with docosahexaenoylcarnitine. With mitochondria from growth hormone-treated rats, rates in the absence of oxaloacetate are positively correlated with the degree of unsaturation of the substrate (see Table II). The addition of oxaloacetate stimulated the oxidation of oleoylcarnitine most and that of docosahexaenoylcarnitine least with the result that rates with all substrates were very similar (see Table II). The observation that docosahexaenoylcarnitine was the best substrate in the absence of oxaloacetate prompts the suggestions that its oxidation yields less NADH or results in the reoxidation of NADH, possibly

TABLE II

EFFECTS OF HYPOPHYSECTOMY AND GROWTH HORMONE TREATMENT ON ROTENONE-INHIBITED RAT LIVER MITOCHONDRIA IN THE PRESENCE AND ABSENCE OF OXALOACETATE[a]

	Rate of β-oxidation (nmol/min/mg protein)[b]			
	Hypophysectomized		Growth hormone	
Acylcarnitine	−OAA[c]	+OAA[c]	−OAA[c]	+OAA[c]
Oleoylcarnitine	14 ± 3	50 ± 4	18 ± 2	80 ± 4
Linoleoylcarnitine	13 ± 4	39 ± 3	24 ± 2	100 ± 5
Linolenoylcarnitine	18 ± 4	42 ± 2	33 ± 4	104 ± 6
Arachidonoylcarnitine	14 ± 2	30 ± 5	42 ± 6	98 ± 10
Docosahexaenoylcarnitine	14 ± 1	15 ± 2	69 ± 7	85 ± 6

[a] Rates of β-oxidation were determined spectrophotometrically by measuring rates of ferricyanide reduction as described by Osmundsen (16).

[b] Values are means ± SD. Samples from at least five different animals were assayed in triplicate each.

[c] In the absence of oxaloacetate (OAA) rotenone-insensitive rates are obtained. Addition of oxaloacetate removes the rotenone-imposed block of β-oxidation.

because of the utilization of NADPH for the reductive removal of double bonds. If so, the increased oxidation rates of polyunsaturated fatty acylcarnitines in mitochondria from animals treated with growth hormone may be the consequence of an increased activity of 2,4-dienoyl-CoA reductase.

To test this hypothesis we assayed the activities of several β-oxidation enzymes, including 2,4-dienoyl-CoA reductase, in mitochondria and peroxisomes isolated from normal rats, hypophysectomized rats, and growth hormone-treated rats. As shown in Table III, the specific activity of the mitochondrial 2,4-dienoyl-CoA reductase is slightly reduced in hypophysectomized rats, but increased threefold over normal rats upon growth hormone treatment. In contrast, the specific activity of the peroxisomal 2,4-dienoyl-CoA reductase is little affected by growth hormone. However, based on a qualitative evaluation of electron micrographs the number of peroxisomes is increased as a result of growth hormone treatment of hypophysectomized rats (data not shown). The specific activities of butyryl-CoA dehydrogenase, acyl-CoA dehydrogenase, and peroxisomal as well as mitochondrial thiolases and 3-hydroxyacyl-CoA dehydrogenases are insignificantly affected by hypophysectomy or growth hormone treatment. The increased specific activity of mitochondrial 2,4-dienoyl-CoA reductase in response to growth hormone treatment seems to be responsible for the higher oxidation rates of polyunsaturated fatty acids.

As a further test for the suggested correlation between the specific activity of 2,4-dienoyl-CoA reductase and oxidation rates of polyunsaturated fatty acids, we have studied how 4-pentenoic acid affects acylcarnitine-supported respiration in mitochondria from hypophysectomized rats and growth hormone-treated rats. 4-Pentenoic acid was chosen for this study because it is metabolized by two pathways (24). The minor pathway yields the metabolite 3-keto-4-pentenoyl-CoA, which inhibits 3-ketoacyl-CoA thiolase and thereby β-oxidation, whereas the metabolites of the major pathway, which involves 2,4-dienoyl-CoA reductase, have no effect on β-oxidation (24). The fraction of 4-pentenoic acid metabolized via the minor pathway and the resulting inhibition of β-oxidation are inversely related to the mitochondrial 2,4-dienoyl-CoA reductase activity. Shown in Fig. 1 is the strong inhibition of linolenoylcarnitine-supported respiration by pentenoic acid in mitochondria from hypophysectomized rats. This result is similar to results obtained with mitochondria

TABLE III

SPECIFIC ACTIVITIES OF SEVERAL ENZYMES OF FATTY ACID OXIDATION IN LIVER MITOCHONDRIA (M) AND PEROXISOMES (P) FROM CONTROL RATS, HYPOPHYSECTOMIZED RATS, AND GROWTH HORMONE-TREATED RATS

	Specific activity (nmol/min/mg protein)[a]					
	Control		Hypophysectomized		Growth hormone	
Enzyme	M	P	M	P	M	P
2,4-Dienoyl-CoA reductase	38 ± 6	22 ± 4	26 ± 7	20 ± 5	112 ± 10	28 ± 8
Palmitoyl-CoA dehydrogenase	18 ± 6	—	12 ± 5	—	26 ± 8	—
Butyryl-CoA dehydrogenase	20 ± 5	—	15 ± 4	—	25 ± 8	—
3-Hydroxyacyl-CoA dehydrogenase	1300 ± 80	260 ± 60	1380 ± 110	250 ± 80	1550 ± 140	300 ± 100
Acetoacyl-CoA thiolase	480 ± 60	80 ± 20	430 ± 50	60 ± 20	530 ± 80	100 ± 40

[a] Enzymes were assayed as described under Experimental Procedures. Values are the means ± SD. Samples from six different animals were assayed in triplicate each.

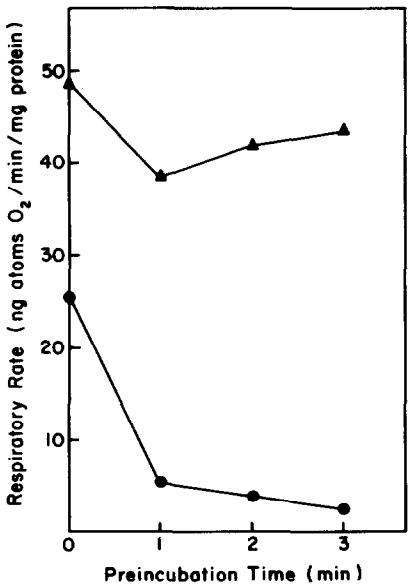

FIG. 1. Effect of 4-pentenoic acid on the rates of mitochondrial respiration supported by linolenoylcarnitine. Liver mitochondria from hypophysectomized rats (●) and hypophysectomized rats treated with growth hormone (▲) were incubated with 0.1 mM 4-pentenoic acid for the indicated periods of time before rates of respiration were measured with 30 μM linolenoylcarnitine in the presence of 5 mM malonate as described under Experimental Procedures.

from normal rats (25). However, when mitochondria from hypophysectomized rats treated with growth hormone were used, little inhibition of respiration was observed. Clearly, growth hormone treatment, which results in a higher specific activity of 2,4-dienoyl-CoA reductase, causes also an increased percentage of 4-pentenoic acid to be oxidized by the major pathway.

Since 2,4-dienoyl-CoA reductase requires NADPH as a coenzyme, we determined how the addition of glutamate, which can reduce $NADP^+$ in the presence of glutamate dehydrogenase affects acetoacetate formation from several acylcarnitines. 3-Hydroxybutyrate was not measured because it is not formed under conditions used to perform this experiment (9). As shown in Table IV glutamate itself yields little acetoacetate. With palmitoylcarnitine and oleoylcarnitine as substrates, glutamate had virtually no effect on the formation of acetoacetate in mitochondria from hypophysectomized rats as well as growth hormone-treated rats. However, with linolenoylcarnitine as a substrate glutamate stimulated acetoacetate formation in mitochondria from both hypophysectomized rats and growth hormone-treated rats. The higher rates of acetoacetate formation from linolenoylcarnitine in growth hormone-treated rats vs hypophysectomized rats in both the absence and the presence of glutamate presumably reflect the higher activity of 2,4-dienoyl-CoA reductase in the former animals.

DISCUSSION

Since acylcarnitine-supported respiration in rat liver mitochondria in the presence of malonate is a measure of fatty acid oxidation, the data presented in Table I demonstrate that highly polyunsaturated

TABLE IV

FORMATION OF ACETOACETATE IN LIVER MITOCHONDRIA FROM HYPOPHYSECTOMIZED AND GROWTH HORMONE-TREATED RATS

Addition[b]	Rate of acetoacetate formation (nmol/min/mg protein)[a]	
	Hypophysectomized	Growth hormone
Glutamate	2.0 ± 0.5	2.2 ± 0.6
Palmitoylcarnitine	18.2 ± 1.4	13.0 ± 0.8
Palmitoylcarnitine + glutamate	18.0 ± 1.2	13.6 ± 0.6
Oleoylcarnitine	12.6 ± 1.2	12.2 ± 0.7
Oleoylcarnitine + glutamate	12.8 ± 1.5	14.1 ± 0.7
Linolenoylcarnitine	3.8 ± 0.4	9.2 ± 0.7
Linolenoylcarnitine + glutamate	8.6 ± 1.0	16.7 ± 0.6

[a] Measurements were performed as described under Experimental Procedures. Values are means ± SD. Samples from at least three different animals were assayed in triplicate each.

[b] Concentrations of glutamate and acylcarnitines were 6 mM and 30 μM, respectively.

fatty acids are oxidized more slowly than are palmitic acid or oleic acid. The slower oxidation of arachidonic acid or docosahexaenoic acid as compared to that of oleic acid could be a consequence of their longer chain lengths or their higher degree of unsaturation. Evidence presented by Osmundsen *et al.* (17) suggests that the removal of double bonds extending from even-numbered carbon atoms may be rate limiting in the degradation of highly polyunsaturated fatty acids because the activity of 2,4-dienoyl-CoA reductase and/or the concentration of NADPH are suboptimal in rat liver mitochondria.

Hypophysectomy has little effect on the rates of respiration supported by various acylcarnitines. Whereas the rates with palmitoylcarnitine and oleoylcarnitine are increased, rates with polyunsaturated acylcarnitines are slightly lower than in control animals. Growth hormone treatment of hypophysectomized rats, however, results in significant increases in ADP-stimulated respiration when polyunsaturated fatty acylcarnitines serve as substrates. Most pronounced is the effect with docosahexaenoylcarnitine as a substrate which was oxidized 3.5 times faster by mitochondria from growth hormone-treated rats than by mitochondria from control rats. If growth hormone stimulates the oxidation of polyunsaturated fatty acylcarnitines by enhancing the mitochondrial capacity to remove double bonds extending from even-numbered carbon atoms, it may do so by inducing the activity of mitochondrial 2,4-dienoyl-CoA reductase and/or by increasing the capacity of mitochondria to generate NADPH. The metabolism of double bonds extending from odd-numbered carbon atoms is probably not rate limiting because of the high activity of *cis*-Δ^3-*trans*-Δ^2-enoyl-CoA isomerase in mitochondria (26).

Under the conditions at which these experiments were performed mitochondrial NADPH is most likely formed from NADH and $NADP^+$ by transhydrogenase. Since the activity of transhydrogenase is increased threefold by growth hormone treatment of hypophysectomized rats (3), the resulting higher potential for generating NADPH could be the cause for the increased oxidation of polyunsaturated fatty acids. The decreased rates of oxidation observed with arachidonoylcarnitine and docosahexaenoylcarnitine in uncoupled mitochondria are most likely a consequence of the lower mitochondrial concentrations of NADH and NADPH. Substitution of ATP for ADP in respiration measurements with coupled mitochondria results in lower rates of oxidative phosphorylation and consequently in higher mitochondrial concentrations of NADH and NADPH. The higher concentration of NADH will inhibit the oxidation of saturated and monosaturated fatty acids. However, the utilization of NADPH in the degradation of polyunsaturated fatty acids promotes the oxidation of NADH and consequently that of fatty acylcarnitine.

The rates at which different acylcarnitines are oxidized were determined by measuring the rates of ferricyanide reduction in the presence of rotenone. In the absence of oxaloacetate NADH is not reoxidized and hence all rates of acylcarnitine oxidation are equally low in hypophysectomized rats. Addition of oxaloacetate stimulates the oxidation of all acylcarnitines with the exception of docosahexaenoylcarnitine. In mitochondria from growth hormone-treated rats the oxidation of polyunsaturated acylcarnitines in the absence of oxaloacetate was higher than in hypophysectomized rats. Docosahexaenoylcarnitine was oxidized most rapidly. Presumably, the reductive removal of double bonds with NADPH leads to the formation of $NADP^+$ and thus of NAD^+ thereby stimulating β-oxidation. In the presence of oxaloacetate all rates of oxidation are increased when compared to rates obtained with mitochondria from hypophysectomized rats. However, the oxidation of docosahexaenoylcarnitine is stimulated much more than is the oxidation of oleoylcarnitine. All observations agree with the hypothesis that the activity of 2,4-dienoyl-CoA reductase and/or the mitochondrial concentration of NADPH is increased by growth hormone treatment.

Assays of 2,4-dienoyl-CoA reductase and several other enzymes of β-oxidation revealed that only the reductase activity is significantly changed in response to treatment with growth hormone. The observed threefold activity increase of 2,4-dienoyl-CoA reductase in mitochondria, but not in peroxisomes, in response to growth hormone treatment equals the stimulation of the oxidation of polyunsaturated fatty acylcarnitines by the same hormone.

The question remained whether the activity of 2,4-dienoyl-CoA reductase is increased in undamaged mitochondria from growth hormone-treated rats. To answer this question we have studied the effect of 4-pentenoic acid on the rate of respiration in coupled liver mitochondria. 4-Pentenoic acid was chosen because its effectiveness as an inhibitor of β-oxidation is inversely related to the activity of 2,4-dienoyl-CoA reductase. This situation is a consequence of the metabolism of 4-pentenoic acid by two competing pathways. The flux through the major pathway is proportional to the activity of 2,4-dienoyl-CoA reductase, whereas the minor pathway yields a metabolite, 3-keto-4-pentenoyl-CoA, which inhibits thiolase and hence β-oxidation (24). The observed inhibition of β-oxidation by 4-pentenoate in mitochondria from hypophysectomized rats, in contrast to virtually no effect in mitochondria from growth hormone-treated rats, supports the conclusion that the capacity of the major pathway and thus of 2,4-dienoyl-CoA reductase is increased in whole mitochondria as a result of growth hormone treatment.

Finally, we evaluated the effect of glutamate, which reduces $NADP^+$ in the presence of glutamate dehydrogenase, on the rate of acetoacetate formation from various fatty acylcarnitines. Of the acylcarnitines tested only linolenoylcarnitine was more rapidly converted to acetoacetate in the presence of glutamate. Since this was observed with mitochondria from hypophysectomized rats and growth hormone-treated rats, it appears that the availability of NADPH restricts the degradation of polyunsaturated fatty acids under the conditions of this experiment.

All observations together lead us to conclude that the rate at which highly polyunsaturated fatty acids are oxidized in rat liver mitochondria is limited by the availability of NADPH and the activity of 2,4-dienoyl-CoA reductase. It thus appears that the removal of double bonds extending from even-numbered carbon atoms may be under some conditions the rate-limiting step in the oxidation of polyunsaturated fatty acids. Furthermore, we show here that the activity of 2,4-dienoyl-CoA reductase in mitochondria, but not in peroxisomes, is subject to regulation by growth hormone. It remains to be established whether growth hormone affects also the biosynthesis of prostaglandins and leukotrienes, possibly as a consequence of its effect on the oxidation of polyunsaturated fatty acids.

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