Increase of the activity state and loss of total activity of the branched-chain 2-oxo acid dehydrogenase in rat diaphragm during incubation

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Actual and total branched-chain 2-oxo acid dehydrogenase activities were determined in homogenates of incubated diaphragms from fed and starved rats. Incubation in Krebs–Ringer buffer increased the activity state, but caused considerable loss of total activity. Palmitate oxidation rates and citrate synthase activities did not significantly change on incubation. Starved muscles showed a higher extent of activation after 15 min of incubation (not after 30 and 60 min) and a smaller loss of total activity. Experiments with the transaminase inhibitor amino-oxyacetate confirm that the contribution of endogenous amino acids to the oxidation precursor pool is also smaller in diaphragms from starved rats on incubation *in vitro*. These phenomena together cause the higher $^{14}\text{CO}_2$ production from ^{14}C -labelled branched-chain amino acids and 2-oxo acids in muscles from starved than from fed rats. High concentrations of branched-chain 2-oxo acids, and the presence of 2-chloro-4-methylpentanoate, octanoate or ketone bodies, increase the extent of activation of the dehydrogenase complex; glucose and pyruvate had no effect. The observed changes of the activity state by these metabolites are discussed in relation to their interaction with branched-chain 2-oxo acid oxidation in incubated hemidiaphragms.

The branched-chain 2-oxo acid dehydrogenase complex, which catalyses the rate-limiting oxidative-decarboxylation step in the degradation of leucine, isoleucine and valine, is subject to covalent modification *in vitro*, with inactivation by phosphorylation and activation by dephosphorylation. Incubation of mitochondria from rat heart and skeletal muscle and perfusion of rat hearts without substrate and with branched-chain 2-oxo acids resulted in a rapid activation of the enzyme complex (reviewed by Randle, 1983). More recently we developed a method to define the activity state of the branched-chain 2-oxo acid dehydrogenase complex in rat tissues (Wagenmakers *et al.*, 1984a). In the present paper we apply this assay in a slightly modified form to rat diaphragms incubated in Krebs–Ringer bicarbonate buffer.

Actual branched-chain 2-oxo acid dehydrogenase activities were similar in diaphragms from fed and starved rats (Wagenmakers *et al.*, 1984b). Oxidative-decarboxylation rates of branched-chain amino acids and 2-oxo acids were much higher, however, in hemidiaphragms *in vitro* from starved than from fed rats (Wagenmakers &

Veerkamp, 1984a). A differential contribution of endogenous amino acids to the oxidation precursor pool in muscles from fed and starved rats was suggested to be the main factor causing the increased oxidation *in vitro* (Wagenmakers & Veerkamp, 1984a). In the present study we obtain information on differences in activation during incubation *in vitro* of diaphragms from fed and starved rats. We also investigate the effect of branched-chain 2-oxo acids, glucose, pyruvate, ketone bodies, octanoate and the metabolic agents amino-oxyacetate and DL-2-chloro-4-methylpentanoate on the interconversion *in vitro* of the branched-chain 2-oxo acid dehydrogenase complex in this muscle system.

Materials and methods

L-[1- ^{14}C]Leucine was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. 4-Methyl-2-oxo[1- ^{14}C]pentanoate was prepared as described by Rüdiger *et al.* (1972). DL-2-Chloro-4-methylpentanoate was a gift from Dr. Ronald Simpson, Sandoz Inc., East Hanover, NJ, U.S.A., and L-carnitine was a gift from Sigma Tau, Rome, Italy. CoA (lithium salt) and thiamin pyrophos-

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phate were from Sigma, St. Louis, MO, U.S.A., and ADP and NAD⁺ (grade 1) were from Boehringer, Mannheim, West Germany. All other chemicals were of the highest purity commercially available.

Male albino Wistar rats (body wt. 110–120g) were fed on a commercial stock diet (RMH-TM from Hope Farms BV, Woerden, The Netherlands) and had free access to water. Rats were used in the fed state or after 3 days of starvation. During starvation they received only water and lost about 25% of their initial body weight. The rats were killed by cervical dislocation. Isolation and preparation of hemidiaphragms have been described previously (Wagenmakers & Veerkamp, 1982).

Both hemidiaphragms isolated from one rat were incubated together for 15, 30 or 60 min at 37°C in 4 ml of Krebs–Ringer bicarbonate buffer (118.5 mM-NaCl, 4.74 mM-KCl, 2.54 mM-CaCl₂, 1.18 mM-KH₂PO₄, 1.18 mM-MgSO₄ and 25.0 mM-NaHCO₃, pH 7.4), gassed with O₂/CO₂ (19:1) and containing substrates and additions as indicated in the Tables. Immediately after the incubation the hemidiaphragms were transferred to ice-cold buffer, consisting of 0.25 M-sucrose, 2 mM-EDTA and 10 mM-Tris/HCl (pH 7.4), and a homogenate (5%, w/v) was prepared in the same buffer by the procedure described by Wagenmakers *et al.* (1984a). To assay the total activity of the branched-chain 2-oxo acid dehydrogenase complex, 100 µl of the homogenate was added to 300 µl of a medium containing 16.7 mM-KH₂PO₄, 25.0 mM-NaHCO₃, 100 mM-Tris/HCl (pH 7.4), 41.7 mM-sucrose, 25.0 mM-KCl, 8.33 mM-EDTA, 1.67 mM-2-oxo-glutarate, 8.33 mM-ADP and 3.33 mM-L-carnitine. To assay the actual activity, 100 µl of the homogenate was added to 300 µl of the same medium containing, in addition 83.3 mM-NaF. After a pre-incubation for 5 min at 37°C, 100 µl of 0.5 mM-4-methyl-2-oxo[1-¹⁴C]pentanoate was added (to measure actual activity) or 100 µl of 0.5 mM-4-methyl-2-oxo[1-¹⁴C]pentanoate/250 mM-NaF (for total activity). After incubation for 5 min at 37°C, reactions were terminated and ¹⁴CO₂ was collected and measured as described previously (Veerkamp *et al.*, 1980). Activities were calculated from the specific radioactivity in the medium (3000 d.p.m./nmol) and were expressed in nmol of 4-methyl-2-oxopentanoate decarboxylated/min per g of diaphragm. Activities were determined in triplicate and were constant for at least 5 min. A blank correction was subtracted (incubation without homogenate). Palmitate oxidation rates were assayed as described by Veerkamp *et al.* (1983). Citrate synthase activity was assayed in the 10000g supernatant from sonicated homogenates as described by Shepherd & Garland (1969).

Results are shown as means ± s.d., with the

numbers of experiments (*n*) in parentheses. Student's unpaired *t* test was used for the statistical analysis of the data.

Results

When the procedure of Wagenmakers *et al.* (1984a) is applied to assay the activity state of the branched-chain 2-oxo acid dehydrogenase complex in rat diaphragm, one hemidiaphragm is homogenized in the presence of inhibitors of the kinase and the phosphatase of the complex and assayed to determine the actual activity. The other is homogenized in a buffer without inhibitors and assayed to determine the total activity. The activity states found with this procedure were rather variable, however, with hemidiaphragms which were first subjected to an incubation in Krebs–Ringer bicarbonate buffer (Table 1). The reason for the large variability may be the induction of unequal changes in the individual hemidiaphragms. We therefore modified the procedure. Actual and total activities were measured as described in the Materials and methods section in one homogenate prepared without inhibitors from both hemidiaphragms. With diaphragms freshly isolated from fed rats, the modified procedure gave a comparable actual activity, but a higher total activity (cf. Tables 1 and 2). Since the assay for the total activity is identical, however, in both cases, this difference is probably due to experimental and/or biological variations. With incubated diaphragms both procedures gave comparable

Table 1. Effect of incubation on the activity of the branched-chain 2-oxo acid dehydrogenase complex in rat diaphragm

Diaphragms from fed rats were incubated for the indicated time in Krebs–Ringer bicarbonate buffer. After that time one hemidiaphragm was homogenized to assay the actual activity and the other to assay the total activity as described by Wagenmakers *et al.* (1984a). Values are means ± s.d. Activities are given in nmol/min per g of muscle. The activity state (actual activity as % of total) and the numbers of experiments (*n*) are given in parentheses. ^{a,b,c}Significantly different from the unincubated control at *P* < 0.001, < 0.01 and < 0.05 respectively.

Incubation time (min)	Actual activity	Total activity
0	7.8 ± 2.3	38.3 ± 9.3 (20 ± 3; <i>n</i> = 5)
15	13.1 ± 1.7 ^b	37.1 ± 5.1 (37 ± 9 ^b ; <i>n</i> = 6)
30	11.9 ± 2.1 ^b	24.4 ± 6.8 ^c (53 ± 20 ^b ; <i>n</i> = 7)
60	7.2 ± 2.1	13.0 ± 4.2 ^a (59 ± 18 ^b ; <i>n</i> = 6)

Table 2. *Effect of incubation on the activity of the branched-chain 2-oxo acid dehydrogenase complex in diaphragm from fed and starved rats*

Diaphragms from fed and 3-days-starved rats were incubated for the indicated time in Krebs–Ringer bicarbonate buffer. After that time a homogenate was prepared and the actual and total activities of the branched-chain 2-oxo acid dehydrogenase complex were assayed as indicated in the Materials and methods section. Values are means $\pm$ S.D. Activities are given in nmol/min per g of muscle. The activity state (actual activity as % of total) and the numbers of experiments (*n*) are given in parentheses. ^{abc}Significantly different from the unincubated control at $P < 0.001$, < 0.01 and < 0.05 respectively. ^{de}Significantly different from the comparable value obtained with diaphragms from fed rats at $P < 0.01$ and < 0.05 respectively.

Incubation time (min)	Fed		Starved	
	Actual	Total	Actual	Total
0	6.7 $\pm$ 2.0	55.2 $\pm$ 11.4 (12 $\pm$ 2; <i>n</i> = 5)	4.1 $\pm$ 1.1 ^e	31.4 $\pm$ 10.5 ^d (14 $\pm$ 5; <i>n</i> = 5)
15	13.2 $\pm$ 2.2 ^b	41.9 $\pm$ 9.3 (32 $\pm$ 3 ^a ; <i>n</i> = 5)	14.6 $\pm$ 4.3 ^b	31.8 $\pm$ 9.5 (47 $\pm$ 6 ^{ad} ; <i>n</i> = 4)
30	8.6 $\pm$ 2.4	17.0 $\pm$ 6.4 ^a (53 $\pm$ 9 ^a ; <i>n</i> = 6)	11.9 $\pm$ 1.1 ^{ae}	28.4 $\pm$ 2.6 ^e (42 $\pm$ 2 ^{ae} ; <i>n</i> = 4)
60	4.3 $\pm$ 0.8 ^c	9.5 $\pm$ 1.2 ^a (46 $\pm$ 8 ^a ; <i>n</i> = 6)	6.5 $\pm$ 1.9 ^{ce}	13.2 $\pm$ 3.1 ^{be} (49 $\pm$ 7 ^a ; <i>n</i> = 6)

Table 3. *Effect of 4-methyl-2-oxopentanoate addition to the medium on the activity of the branched-chain 2-oxo acid dehydrogenase complex in incubated rat diaphragm*

Diaphragms from fed and 3-days-starved rats were incubated for the indicated time in Krebs–Ringer bicarbonate buffer. The concentration of 4-methyl-2-oxopentanoate was 0.1 mM and of amino-oxyacetate 1.0 mM. Other details are given in the legend of Table 2. ^{abc}Significantly different from the appropriate control (without addition; see Table 2) at $P < 0.001$, < 0.01 and < 0.05 respectively. ^{def}Significantly different from the comparable value obtained with hemidiaphragms from fed rats at $P < 0.001$, < 0.01 and < 0.05 respectively.

Incubation time (min)	Addition	Fed		Starved	
		Actual	Total	Actual	Total
15	4-Methyl-2-oxopentanoate	12.8 $\pm$ 2.8	38.9 $\pm$ 3.3 (33 $\pm$ 7; <i>n</i> = 7)	15.7 $\pm$ 0.9	35.7 $\pm$ 4.6 (44 $\pm$ 4 ^f ; <i>n</i> = 4)
60	4-Methyl-2-oxopentanoate	5.6 $\pm$ 1.2	10.7 $\pm$ 2.6 (53 $\pm$ 4; <i>n</i> = 4)	9.8 $\pm$ 2.8 ^{cf}	18.1 $\pm$ 4.7 ^f (54 $\pm$ 3; <i>n</i> = 6)
15	4-Methyl-2-oxopentanoate and amino-oxyacetate	17.8 $\pm$ 1.5 ^b	39.2 $\pm$ 6.2 (46 $\pm$ 6 ^b ; <i>n</i> = 5)	30.5 $\pm$ 1.3 ^{ad}	49.4 $\pm$ 7.4 ^a (63 $\pm$ 8 ^{ce} ; <i>n</i> = 4)
60	4-Methyl-2-oxopentanoate and amino-oxyacetate	9.5 $\pm$ 1.7 ^a	14.9 $\pm$ 2.3 ^b (64 $\pm$ 8 ^b ; <i>n</i> = 4)	12.0 $\pm$ 2.4 ^b	20.3 $\pm$ 3.4 ^{bf} (60 $\pm$ 9; <i>n</i> = 4)

mean values for the activity state, whereas the variability was considerably smaller with the modified procedure (cf. Tables 1 and 2).

Incubation of diaphragms from fed and starved rats in Krebs–Ringer bicarbonate buffer increased the activity state of the branched-chain 2-oxo acid dehydrogenase complex and caused loss of total activity, especially after incubations longer than 15 min (Table 2). The addition of NAD⁺, CoA and thiamin pyrophosphate (1 mM each) increased the actual and total activities measured after 60 min of incubation only by 52 $\pm$ 10 and 35 $\pm$ 8% (*n* = 4; fed rats), respectively. In diaphragm homogenates from fed rats, total palmitate oxidation rates did not change significantly on incubation for 60 min (273 $\pm$ 32 and 244 $\pm$ 9 nmol/

min per g of muscle respectively; *n* = 4), nor did citrate synthase activities (27.7 $\pm$ 5.1 and 26.3 $\pm$ 1.3 μ mol/min per g of muscle respectively; *n* = 4). The marked loss of total branched-chain 2-oxo acid dehydrogenase activity therefore appears to be a specific deterioration of this mitochondrial activity.

A higher activity state and a loss of total activity were also observed after incubation in the presence of 0.1 mM 4-methyl-2-oxopentanoate (Table 3). In comparison with incubations without substrate (Table 2), this concentration of 4-methyl-2-oxopentanoate hardly had any effect on the measured activities. Simultaneous addition of 4-methyl-2-oxopentanoate and amino-oxyacetate caused a higher activity state both after 15 and 60 min of

incubation and decreased the loss of total activity in muscles from fed and starved rats (Table 3). In all cases (Tables 2 and 3) the conversion of inactive into active complex was larger after 15 min of incubation in diaphragms from starved rats, but the same activity state was reached after 60 min of incubation. In all cases the loss of total activity on incubation appeared to be smaller in muscles from starved than from fed rats.

Addition of 1.0 mM 4-methyl-2-oxopentanoate increased the activity state after 15 and 60 min of incubation and slightly increased the total activity present after 60 min of incubation (Table 4). Addition of 0.1 mM- and 1.0 mM-3-methyl-2-oxobutanoate had little effect; the higher 2-oxo acid concentration slightly increased the activity state and the total activity present after 60 min of incubation.

DL-2-Chloro-4-methylpentanoate inhibits the branched-chain 2-oxo acid dehydrogenase kinase reaction (Harris *et al.*, 1982a). This metabolic agent increased the $^{14}\text{CO}_2$ production from ^{14}C -labelled branched-chain 2-oxo acids (Wagenmakers & Veerkamp, 1984b) and caused a rapid increase of the activity state to about 75% (Table 4).

Glucose (4 mM and 10 mM) and pyruvate (2 mM)

had no effect on the activities measured after incubation (Table 4). Glucose also had no effect on branched-chain 2-oxo acid oxidation by rat hemidiaphragms, but pyruvate inhibited this process, probably by competition for mitochondrial uptake (Wagenmakers & Veerkamp, 1984b).

3-Hydroxybutyrate and acetoacetate were tested together in the indicated ratio (Table 4) to minimize disturbances of the intracellular redox state (Zapalowski *et al.*, 1981; Snell & Duff, 1982). Although the ketone bodies inhibit branched-chain 2-oxo acid oxidation by rat hemidiaphragms (Wagenmakers & Veerkamp, 1984b), they increased the activity state present after 15 min and 60 min of incubation (Table 4). It therefore appears plausible that ketone bodies inhibit branched-chain 2-oxo acid oxidation in rat hemidiaphragms, just like pyruvate does, by competition for mitochondrial uptake. Ketone bodies can enter mitochondria by diffusion as undissociated acids and as anions with a carrier similar to or identical with that used by pyruvate (Robinson & Williamson, 1980).

Octanoate stimulated branched-chain 2-oxo acid oxidation in intact rat skeletal muscle *in vitro* (Wagenmakers & Veerkamp, 1984c). In agreement with this observation, octanoate increased the

Table 4. Effect of substrate addition to the medium on the activity of the branched-chain 2-oxo acid dehydrogenase complex in incubated rat diaphragm

Diaphragms from fed rats were incubated for 15 or 60 min in Krebs-Ringer bicarbonate buffer, containing the indicated additions. Other details are given in the legend of Table 2. ^{abc}Significantly different from the control (without addition) at $P < 0.001$, < 0.01 and < 0.05 respectively. N.D., not determined.

Addition	Concn. (mM)	15 min incubation		60 min incubation	
		Actual	Total	Actual	Total
-	-	13.2 ± 2.2	41.9 ± 9.3 (32 ± 3; n = 5)	4.3 ± 0.8	9.5 ± 1.2 (46 ± 8; n = 6)
4-Methyl-2-oxopentanoate	1.0	24.9 ± 7.5 ^c	36.0 ± 9.6 (69 ± 4 ^a ; n = 4)	10.2 ± 1.1 ^a	12.7 ± 1.1 ^b (80 ± 2 ^a ; n = 4)
3-Methyl-2-oxobutanoate	0.1	11.7 ± 2.2	36.9 ± 1.8 (32 ± 7; n = 4)	N.D.	N.D.
	1.0	14.9 ± 3.3	37.5 ± 4.4 (39 ± 6; n = 4)	8.9 ± 1.7 ^a	15.4 ± 3.6 ^b (58 ± 4 ^c ; n = 4)
DL-2-Chloro-4-methylpentanoate	0.5	27.9 ± 4.2 ^a	37.4 ± 3.4 (75 ± 7 ^a ; n = 4)	7.5 ± 3.7	10.2 ± 4.9 (73 ± 2 ^a ; n = 4)
Pyruvate	2.0	11.2 ± 0.8	33.1 ± 1.5 (34 ± 3; n = 4)	N.D.	N.D.
Glucose	4.0	12.0 ± 1.8	33.6 ± 5.4 (36 ± 2; n = 4)	N.D.	N.D.
	10.0	N.D.	N.D.	5.6 ± 1.9	12.8 ± 4.5 (44 ± 2; n = 4)
DL-3-Hydroxybutyrate and acetoacetate	4.0	12.8 ± 1.3	26.3 ± 3.2 ^c (49 ± 5 ^a ; n = 4)	4.5 ± 1.0	7.5 ± 2.3 (63 ± 13 ^c ; n = 4)
Octanoate	1.0	24.4 ± 3.0 ^a	37.1 ± 3.2 (66 ± 5 ^a ; n = 4)	9.5 ± 0.8 ^a	11.3 ± 0.8 ^c (84 ± 3 ^a ; n = 4)
3-Methyl-2-oxobutanoate and octanoate	0.1	25.8 ± 6.4 ^b	36.3 ± 8.5 (71 ± 5 ^a ; n = 4)	N.D.	N.D.
	1.0				

activity states measured after 15 and 60 min of incubation (Table 4). The simultaneous presence of octanoate (1 mM) and 3-methyl-2-oxobutanoate (0.1 mM) had the same effect as octanoate alone.

Discussion

The results from the present study show that hemidiaphragms incubated *in vitro* are not an adequate system in which to measure oxidation rates of branched-chain amino acids and 2-oxo acids, although the time courses of $^{14}\text{CO}_2$ production from 4-methyl-2-oxo-[1- ^{14}C]pentanoate were linear for 60 min or more (Wagenmakers & Veerkamp, 1982, 1984a). Regarding the results in Tables 1–4, it is evident, however, that this linearity is only apparent. Loss of total activity is more or less compensated by conversion of inactive into active complex. The measured rates therefore will not reflect activities present *in vivo*. Similar objections can be raised to experiments in which hemidiaphragms have been preincubated before the assay of oxidation (e.g. Buse *et al.*, 1975; Snell & Duff, 1982).

The results given in Table 3 show that the simultaneous presence of 4-methyl-2-oxopentanoate and the transaminase inhibitor amino-oxoacetate increases the activity states present after 15 and 60 min of incubation to a comparable extent in muscles from fed and starved rats. The smaller stimulation by amino-oxoacetate of $^{14}\text{CO}_2$ production rates from ^{14}C -labelled branched-chain 2-oxo acids in muscles from starved than from fed rats (Wagenmakers & Veerkamp, 1984a) therefore cannot result from a different extent of activation of the branched-chain 2-oxo acid dehydrogenase complex. These results sustain our previous conclusion (Wagenmakers & Veerkamp, 1984a) that amino-oxoacetate has less effect on $^{14}\text{CO}_2$ production rates in muscles of 3-day-starved rats because the oxidation precursor pool is less diluted with 2-oxo acids derived from amino acids originating from protein degradation.

The faster conversion of inactive into active complex and the smaller loss of total activity in muscles from starved than from fed rats (Tables 2 and 3) will in part cause the higher $^{14}\text{CO}_2$ production from ^{14}C -labelled branched-chain amino acids and 2-oxo acids in muscles from starved rats *in vitro* (see Wagenmakers & Veerkamp, 1984a). These phenomena do not appear to explain, however, the 3–4 times higher $^{14}\text{CO}_2$ production from 10 μM - and 20 μM 4-methyl-2-oxo-[1- ^{14}C]pentanoate (Wagenmakers & Veerkamp, 1984a). With a 10 μM concentration, this difference was already present after 10 min of incubation [2.8 ± 0.7 ($n = 9$) and 12.5 ± 5.3 ($n = 11$) nmol of $^{14}\text{CO}_2/\text{g}$ in muscles from fed and starved rats

respectively]. At 0–15 min of incubation the actual activity present in muscles from fed and starved rats was, however, of comparable magnitude (Tables 2 and 3). Part of the increased $^{14}\text{CO}_2$ production in muscles from starved rats therefore appears to be due to the smaller contribution of endogenous amino acids to the oxidation precursor pool (Wagenmakers & Veerkamp, 1984a).

Conversion of inactive into active branched-chain 2-oxo acid dehydrogenase complex was also found in rat hearts perfused without substrate, with branched-chain 2-oxo acids and with 2-chloro-4-methylpentanoate (Parker & Randle, 1980; Sans *et al.*, 1980; Waymack *et al.*, 1980; Harris *et al.*, 1982a,b; Paxton & Harris, 1984). In contrast with the results obtained with diaphragm (Table 4), glucose, pyruvate and ketone bodies prevent in the perfused heart the activation observed without substrate. Glucose, was, however, not able to prevent activation by branched-chain 2-oxo acids (Parker & Randle, 1980; Paxton & Harris, 1984) and by 2-chloro-4-methylpentanoate (Harris *et al.*, 1982a).

The regulation of the branched-chain 2-oxo acid dehydrogenase kinase reaction was studied in enzyme complexes, containing intrinsic kinase activity, isolated from ox kidney (Lau *et al.*, 1982) and from rabbit liver (Paxton & Harris, 1984). Both groups found inhibition by ADP and branched-chain 2-oxo acids. These mechanisms may be involved in the conversion of inactive into active complex observed in the present study. The significance of changes in concentrations of ATP and ADP is not clear. ATP concentrations decreased in diaphragms during the first 30 min of incubation (Goldberg *et al.*, 1975). In the presence of glucose, ATP and ADP concentrations did not change, however, during an incubation for 14 min (Bessman & Geiger, 1980), whereas activation still occurred in the presence of glucose (Table 4). The larger extent of activation by 4-methyl-2-oxopentanoate than by 3-methyl-2-oxobutanoate (Table 4) is in agreement with the concentrations of the respective 2-oxo acids giving 40% and 50% inhibition for the kinase reaction (Lau *et al.*, 1982; Paxton & Harris, 1984). The higher concentration of branched-chain 2-oxo acids in muscles from starved than from fed rats (Hutson & Harper, 1981) may play a role in the faster activation in muscles from starved rats (Tables 2 and 3). Acetoacetyl-CoA inhibition of the kinase (Paxton & Harris, 1984) probably causes the activation by ketone bodies (Table 4). Octanoate may also induce conversion into active complex by direct inhibition of the kinase (Paxton & Harris, 1984). It is questionable, however, whether a considerable amount of octanoate will enter the mitochondria in the free acid form in rat hemidiaphragms

incubated with octanoate. Another possibility, therefore, still remains that octanoate decreases the intramitochondrial concentration of branched-chain acyl-CoA esters (Wagenmakers & Veerkamp, 1984c). This decrease may relieve the direct product inhibition of the branched-chain 2-oxo acid dehydrogenase complex (Randle, 1983) and may decrease inactivation of the complex by phosphorylation. High isovaleryl-CoA/CoA ratios accelerate the kinase reaction, according to Lau *et al.* (1982). Paxton & Harris (1984) found, however, inhibition of the kinase reaction by isobutyryl-CoA and isovaleryl-CoA.

In only one study the branched-chain 2-oxo acid dehydrogenase complex was isolated with intrinsic phosphatase activity (Fatania *et al.*, 1983). No information available on its regulation appears to be relevant for the present study.

The rapid and extensive activation *in vitro* contrasts with the marginal differences between the activity states of the branched-chain 2-oxo acid dehydrogenase complex in tissues of fed, starved and exercised rats (Wagenmakers *et al.*, 1984a,b). This suggests the existence *in vivo* of an additional regulatory mechanism which limits interconversion of the complex. Although future experiments have to give evidence, it is tempting to speculate that hormones and neural influences are involved in that kind of regulation. Indications for hormonal regulation of branched-chain amino acid oxidation are given by studies both *in vitro* (Buse *et al.*, 1973, 1976; Hutson *et al.*, 1980; Buxton *et al.*, 1982; Palmer *et al.*, 1983) and *in vivo* (McCallister *et al.*, 1983).

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