The effect of starvation on branched-chain 2-oxo acid oxidation in rat muscle

Anton J. M. WAGENMAKERS and Jacques H. VEERKAMP*

Department of Biochemistry, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

(Received 30 August 1983/Accepted 22 December 1983)

Oxidative-decarboxylation rates of branched-chain amino acids in rat hemidiaphragm and of branched-chain 2-oxo acids in hemidiaphragm, soleus muscle and heart slices of 110–120 g rats were increased considerably by 3–4 days of starvation, when they were calculated from the specific radioactivity in the medium. When the supply from endogenous protein degradation to the oxidation-precursor pool was severely limited by transaminase inhibitors, oxidative-decarboxylation rates of branched-chain 2-oxo acids rose significantly. Since this apparent increase was relatively larger in preparations from fed rats than from 3-days-starved rats, the differences in oxidation rates with nutritional state became less or even not significant. With rat heart the smaller dilution of the oxidation precursor pool after starvation is in accordance with the reported decrease in protein breakdown. Since protein degradation increases with starvation in skeletal muscles, we suggest that the amino acid pool arising from protein degradation is more segregated from the oxidation precursor pool in muscles from starved than from fed rats. We conclude that starvation increases branched-chain amino acid and 2-oxo acid oxidation in skeletal and cardiac muscle considerably less than has been suggested by previous studies.

The liver is the primary site for the degradation of most amino acids, except for the branched-chain amino acids, which are metabolized to a large degree in muscle (Miller, 1962; Shinnick & Harper, 1976; Felig, 1977; Goldberg & Chang, 1978; Veerkamp *et al.*, 1980; Veerkamp & Wagenmakers, 1981; Wagenmakers & Veerkamp, 1982). The first two steps in the degradation route, transamination and oxidative decarboxylation, have been most extensively studied. Oxidative decarboxylation of branched-chain amino acids in muscle is reported or assumed to be increased during starvation (Meikle & Klain, 1972; Goldberg & Odessey, 1972; Adibi *et al.*, 1974; Hutson *et al.*, 1980; Tischler & Goldberg, 1980). There are two sources of branched-chain amino acids in muscle, the external medium and endogenous protein degradation. In most of the earlier studies the contribution of endogenous protein degradation was neglected, and therefore quantitative conclusions regarding the extent of branched-chain amino acid oxidation would be inaccurate.

The present paper re-evaluates the effect of star-

vation on branched-chain amino acid oxidation. The oxidative-decarboxylation rates of branched-chain 2-oxo acids were investigated in intact preparations of skeletal and heart muscles of 110–120 g rats to verify if the increase in $^{14}\text{CO}_2$ production from ^{14}C -labelled branched-chain amino acids represents a real increase in the oxidative-decarboxylation step. There are several ways to get an impression of the contribution of amino acids derived from protein degradation to the oxidation precursor pool. Provided that isotopic equilibrium exists between the branched-chain 2-oxo acid pool and the oxidation-precursor pool, it would be best to measure specific radioactivities of intracellular branched-chain 2-oxo acids. Hutson *et al.* (1980) previously argued, however, that intracellular branched-chain 2-oxo acid concentrations are too low to measure specific radioactivities. For this reason we determined the oxidative-decarboxylation rates at various concentrations and incubation periods and applied the transaminase inhibitors amino-oxyacetate and L-cycloserine, which limit the production of branched-chain 2-oxo acids from amino acids originating from endogenous protein degradation. Furthermore, we measured the trans-

* To whom reprint requests should be addressed.

amination rates of both leucine and 4-methyl-2-oxopentanoate and the incorporation of the 2-oxo acid into protein.

Materials and methods

Amino-oxyacetate and cycloheximide were purchased from Sigma, St. Louis, MO, U.S.A., L-cycloserine was from Fluka A.G., Buchs, Switzerland, and Aquasol scintillation fluid from New England Nuclear, Boston, MA, U.S.A. Sources of other radiochemicals and chemicals used have been reported previously (Wagenmakers & Veerkamp, 1982). Male albino Wistar rats weighing 110–120 g were used, except for the experiments of Table 1 (90–120 g). The rats were fed on a commercial stock diet (RMH-TM, obtained from Hope Farms BV, Woerden, The Netherlands) and water *ad libitum*. During starvation they only received water *ad libitum*. After 3 days of starvation they had lost about 25% of their initial weight. The rats were killed by cervical dislocation. Isolation and preparation of hemidiaphragms, soleus muscles and heart slices have been described previously (Wagenmakers & Veerkamp, 1982).

The intact muscle preparations were incubated for 90 min (unless otherwise indicated) at 37°C in 2 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) containing 4 mM-glucose and 0.5 mM 1-¹⁴C-labelled branched-chain amino acid or 0.1 mM 1-¹⁴C-labelled branched-chain 2-oxo acid (Wagenmakers & Veerkamp, 1982). When the branched-chain 2-oxo acid concentration was decreased to 10 or 20 μM, the incubations were usually terminated after 30 min. The heart slices were incubated in triplicate. Termination of the incubations, collection of ¹⁴CO₂ and chemical decarboxylation of branched-chain 2-oxo acids with ceric sulphate (both with 100% recovery) have been described previously (Wagenmakers & Veerkamp, 1982). Oxidative decarboxylation and transamination rates were calculated from the specific radioactivity in the medium at the start of the experiment (500–3000 d.p.m./nmol) and were expressed in nmol of ¹⁴C-labelled compound metabolized/min per g of muscle. The effects of amino-oxyacetate and L-cycloserine on transamination and oxidative-decarboxylation rates were assayed in two parallel incubations of hemidiaphragms and soleus muscles or two sets of three heart slices obtained from one rat.

The production of leucine from 1-¹⁴C-labelled 4-methyl-2-oxopentanoate and the incorporation of radioactive label into protein was assayed as follows. After termination of the incubations and collection of the released ¹⁴CO₂, the hemidia-

phragms were divided into small pieces with a pair of scissors and disrupted with a Polytron tissue homogenizer (X 1020; Ystral, Dottingen, Germany) in ice-cold 0.6 M-HClO₄-containing incubation medium and an additional 5 ml of 5% (w/v) trichloroacetic acid. The resulting protein precipitate was pelleted and washed with 2 × 5 ml of ice-cold 5% trichloroacetic acid. The combined supernatants were neutralized and passed through a column (1 cm × 10 cm) of Dowex 50 W X2 (H⁺ form; 50–100 mesh). 4-Methyl-2-oxopentanoate was eluted with water, and leucine with 5 M-NH₃, both with 100% recovery. Radioactivity was measured in both fractions. The protein pellet was washed with 5 ml of ethanol/diethyl ether (1:1, v/v) and dissolved in 1 ml of 1 M-NaOH (60 min at 90°C). The dissolved material was transferred with 3 ml more of water to scintillation vials and, after addition of 10 ml of Aquasol, radioactivity was measured. Leucine production and incorporation of label into protein were based on the specific radioactivity of 1-¹⁴C-labelled 4-methyl-2-oxopentanoate added to the incubation medium at the start of the experiment and expressed as nmol of 1-¹⁴C-labelled leucine produced or incorporated per g of muscle during the indicated incubation period.

For protein-degradation studies the total amount of acid-soluble tyrosine was measured in separate incubations in the presence of 0.1 mM non-radioactive 4-methyl-2-oxopentanoate. Cycloheximide (0.5 mM) was added to inhibit protein synthesis. The incubations were terminated after 90 min by the addition of 0.5 ml of 50% trichloroacetic acid. Incubation medium and hemidiaphragms (cut into pieces with a pair of scissors) were transferred to centrifuge tubes and the tissue was further disrupted with a Polytron tissue homogenizer. Protein was pelleted by centrifugation, and acid-soluble tyrosine was measured in the supernatant by the method of Waalkes & Udenfriend (1957).

Student's unpaired or paired *t* test was used for the statistical analysis of the data. Results are shown as means ± S.D., with the numbers of experiments (*n*) in parentheses.

Results

Oxidative-decarboxylation rates of branched-chain amino acids were reported to be increased in skeletal and atrial muscle preparations from starved rats as compared with those from fed rats (Goldberg & Odessey, 1972; Adibi *et al.*, 1974; Hutson *et al.*, 1980; Tischler & Goldberg, 1980). This was attributed to an acceleration of the rate of oxidative decarboxylation. To verify this assumption, we measured ¹⁴CO₂ production in hemidiaphragms from fed and starved rats, not only with 1-

^{14}C -labelled leucine and valine, but also with their derived 2-oxo acids. When calculated on the basis of specific radioactivity of the medium at the start of the incubation, the oxidative-decarboxylation rates of leucine and valine (Table 1) were increased significantly after 1 or more days of starvation. The same holds for the oxidative-decarboxylation rate of 3-methyl-2-oxobutanoate, whereas that of 4-methyl-2-oxopentanoate was only increased after 3 and 4 days of starvation. With the latter, a significant decrease was even found with hemidiaphragms from 1-day-starved rats (Table 1).

The transamination rate of leucine also was increased significantly after 1 or more days of starvation (Table 2). In each nutritional condition tested, transamination rates of leucine were higher than its oxidative-decarboxylation rates (Table 1) and consequently 4-methyl-2-oxopentanoate accumulated. The accumulation was larger during incubations of hemidiaphragms from 1-day-starved rats than from fed and 3-day-starved rats (6.5 ± 0.3 versus 3.4 ± 0.2 and 3.6 ± 1.1 nmol/min per g respectively; $n = 4$). This correlates with the lower oxidative-decarboxylation rate of 4-methyl-2-oxopentanoate in muscles from the former animals. The reverse reaction, transamination of $1\text{-}^{14}\text{C}$ -labelled 4-methyl-2-oxopentanoate taken up from the medium, was measured at concentrations of 10

and $100\text{ }\mu\text{M}$ (Table 2). At $100\text{ }\mu\text{M}$ concentration, production of leucine was proportional to the time of incubation and its rate was of the same order as, or even higher than, the oxidative-decarboxylation rate of the 2-oxo acid (Table 1). There was, however, no difference in transamination rates of 4-methyl-2-oxopentanoate between hemidiaphragms from fed, 1-day-starved and 3-day-starved rats (Table 2).

Since there are no evident reasons to assume that the supply of non-radioactive amino acids from protein degradation is negligible, we tried to block the production of 2-oxo acids from these amino acids with the transaminase inhibitor amino-oxyacetate. At 1 mM, this inhibitor decreased the transamination rates of 0.5 mM -leucine to 25 ± 3 , 21 ± 2 and $24 \pm 2\%$ and the oxidative-decarboxylation rates to 26 ± 4 , 21 ± 3 and $24 \pm 2\%$ in hemidiaphragms from fed, 1-day-starved and 3-day-starved rats respectively ($n = 4$). Raising the amino-oxyacetate concentration to 2 mM decreased the oxidative-decarboxylation rate of leucine further, to $16 \pm 4\%$ of the control ($n = 4$). Furthermore, 1 mM-amino-oxyacetate decreased the reverse transamination of 4-methyl-2-oxopentanoate taken up from the medium, to 28 ± 4 and $31 \pm 6\%$ at $10\text{ }\mu\text{M}$ 2-oxo acid and to 18 ± 5 and $16 \pm 2\%$ at $100\text{ }\mu\text{M}$ in hemidiaphragms from fed and 3-day-

Table 1. Effect of starvation on oxidative-decarboxylation rates of branched-chain amino acids and 2-oxo acids by rat hemidiaphragms

Oxidative-decarboxylation rates are given as means $\pm$ S.D. for the numbers of experiments in parentheses. Concentrations of branched-chain amino and 2-oxo acids were 0.5 mM and 0.1 mM respectively. Abbreviation: ND, not determined. ^{a,b,c} Significantly different from fed animals at $P < 0.001$, < 0.01 and < 0.05 respectively.

Oxidative-decarboxylation rate (nmol/min per g)

1- ^{14}C -labelled substrate	Fed	Starved (days)			
		1	2	3	4
Leucine	9.2 ± 1.7 (12)	11.1 ± 2.1^c (16)	16.0 ± 2.5^a (4)	19.9 ± 3.2^a (7)	27.2 ± 7.9^a (4)
Valine	2.6 ± 0.4 (7)	4.7 ± 1.3^b (9)	5.8 ± 1.0^a (4)	ND	15.4 ± 3.0^a (4)
4-Methyl-2-oxopentanoate	7.5 ± 1.5 (31)	5.7 ± 1.2^a (98)	7.0 ± 1.7 (11)	12.6 ± 2.3^a (20)	17.3 ± 4.1^a (4)
3-Methyl-2-oxobutanoate	$32. \pm 1.0$ (19)	4.0 ± 1.3^c (90)	7.0 ± 2.0^a (4)	8.3 ± 1.9^a (11)	12.1 ± 3.4^a (8)

Table 2. Transamination rates of leucine and 4-methyl-2-oxopentanoate by hemidiaphragms from fed and starved rats

Transamination rates are given as means $\pm$ S.D. for four or five experiments. Abbreviation: ND, not determined.

^{a,b} Significantly different from fed animals at $P < 0.001$ and < 0.01 respectively.

Transamination rate (nmol/min per g)

1- ^{14}C -labelled substrate	Concn. (μM)	Starved (days)		
		Fed	1	3
Leucine	500	11.6 ± 1.9	17.6 ± 1.7^b	25.7 ± 0.2^a
4-Methyl-2-oxopentanoate	10	3.0 ± 0.3	ND	3.1 ± 0.5
4-Methyl-2-oxopentanoate	100	11.6 ± 1.8	9.9 ± 1.0	11.1 ± 2.4

starved rats respectively ($n = 4$). The production of branched-chain 2-oxo acids from amino acids originating from endogenous protein degradation is probably decreased to about the same extent as the transamination of amino acids or 2-oxo acids taken up from the medium. Oxidative-decarboxylation rates of branched-chain 2-oxo acids measured in the presence of amino-oxyacetate consequently will be a better approximation to the real flux *in vitro* through the branched-chain 2-oxo acid dehydrogenase complex.

Addition of amino-oxyacetate to the incubation medium raised the oxidative-decarboxylation rates of branched-chain 2-oxo acids more in hemidiaphragms from fed than from 3-day-starved rats (Table 3). In hemidiaphragms from 1-day-starved rats the inhibitor increased the oxidative-decarboxylation rate of 100 μM -4-methyl-2-oxopentanoate to 174 ± 26 and that of 100 μM -3-methyl-2-oxobutanoate to $176 \pm 27\%$ of the control ($n = 9$ and 7 respectively). The difference in the oxidative-decarboxylation rates between muscle preparations from fed and 3-day-starved rats became much smaller in the presence of the transaminase inhibitor. At 100 μM -4-methyl-2-oxopentanoate this difference became even statistically not significant, whereas it remained significant at the more physiological concentrations of 10 and 20 μM . The residual transaminase activity in the presence of 1 mM-amino-oxyacetate may, however, deliver a relatively larger amount of endogenous 2-oxo acids at the lower 2-oxo acid concentration in the medium.

The time course of oxidative decarboxylation of 0.1 mM-4-methyl-2-oxopentanoate in the presence and absence of amino-oxyacetate with hemidiaphragms from fed and 3-day-starved rats is shown in Fig. 1. Linearity was present from zero time to

60 min, but not to the usual assay time of 90 min, in contrast with results obtained previously with intact muscle preparations from 1-day-starved animals (Wagenmakers & Veerkamp, 1982). The reason for this discrepancy is probably that radioactive substrate becomes limiting earlier owing to the higher oxidative-decarboxylation rates rather than lack of isotopic equilibrium. The same oxidative-decarboxylation rate was measured when the specific radioactivity of 4-methyl-2-oxopentanoate was varied from 500 to 3000 d.p.m./nmol in each nutritional condition. At all incubation times tested, amino-oxyacetate increased the oxidative decarboxylation of 4-methyl-2-oxopentanoate to the same extent in hemidiaphragms from fed rats, but it had no effect with 3-day-starved animals (Fig. 1). This was also found for incubation times of 10, 20 and 30 min with 10 μM -4-methyl-2-oxopentanoate (results not shown).

Incorporation of label from 1- ^{14}C -labelled 4-methyl-2-oxopentanoate into protein (Table 4) amounted to about 5 and 3% of the production of leucine in muscles from fed rats and from 3-day-starved rats respectively (Table 2). Incorporation was significantly lower in muscles from 3-day-starved than from fed rats at 2-oxo acid concentrations of 10, 20 and 100 μM . In muscles from 1-day-starved rats the incorporation was, however, larger than in those from fed rats at 100 μM concentration. At 1 mM, amino-oxyacetate decreased the extent of incorporation to about 40% of the control, independently of the nutritional state and the 2-oxo acid concentration. The inhibitory effect of amino-oxyacetate on the incorporation of label into protein is smaller than on the production of leucine, which was 28–31 and 16–18% of the control at 10 μM - and 100 μM -4-methyl-2-oxopentanoate respectively, independently of the nutritional state.

Table 3. Oxidative-decarboxylation rates of branched-chain 2-oxo acids by hemidiaphragms from fed and starved rats measured in the presence and absence of amino-oxyacetate

Values are given as means $\pm$ S.D. for five to nine experiments. The relative activity in the presence of 1 mM-amino-oxyacetate (as % of the control; mean $\pm$ S.D.) is given in parentheses. ^{a,b,c} Significantly different from the control at $P < 0.001$, < 0.01 and < 0.05 respectively. ^{d,e,f} Significantly different from the comparable incubation with fed animals at $P < 0.001$, < 0.01 and < 0.05 respectively.

1- ^{14}C -labelled substrate	Concn. (μM)	Oxidative-decarboxylation rate (nmol/min per g)			
		Fed		Starved 3 days	
		Control	+ Amino-oxyacetate	Control	+ Amino-oxyacetate
4-Methyl-2-oxopentanoate	10	0.33 ± 0.06	1.06 ± 0.17^a (362 $\pm$ 79)	0.85 ± 0.17^d	$1.39 \pm 0.24^{b,f}$ (176 $\pm$ 10)
	20	0.96 ± 0.23	2.76 ± 0.19^a (298 $\pm$ 59)	2.38 ± 0.43^d	$3.91 \pm 0.70^{b,e}$ (164 $\pm$ 3)
	100	6.5 ± 1.6	12.0 ± 1.8^a (190 $\pm$ 26)	11.1 ± 2.1^d	13.7 ± 1.5^e (125 $\pm$ 18)
3-Methyl-2-oxobutanoate	100	4.3 ± 0.7	8.0 ± 0.7^a (189 $\pm$ 34)	8.4 ± 2.5^e	$11.1 \pm 3.2^{b,f}$ (133 $\pm$ 13)

Fig. 1. Time course of $^{14}\text{CO}_2$ production from 0.1 mM-4-methyl-2-oxo[1- ^{14}C]pentanoate by rat diaphragm. Hemidiaphragms were incubated with (●) or without (○) 1 mM-amino-oxyacetate. Values are means $\pm$ S.D. for four hemidiaphragms obtained from different rats on the same day. With muscles from fed rats (a) values obtained in the presence of amino-oxyacetate were significantly ($P < 0.001$) higher than those obtained in its absence. With muscles from 3-day-starved rats (b) no significant difference was observed.

Table 4. Incorporation of ^{14}C label of 4-methyl-2-oxopentanoate into proteins of hemidiaphragms from fed and starved rats measured in the presence and absence of amino-oxyacetate

Values are means $\pm$ S.D. for four to five experiments. The relative incorporation in the presence of 1 mM-amino-oxyacetate (as % of the control) is given in parentheses. ^a Significantly different from the control at $P < 0.01$. ^b Significantly different from the comparable incubations with fed animals at $P < 0.01$.

Condition	Concn. (μM)	Incubation time (min)	Incorporation (nmol/g)	
			Control	+ Amino-oxyacetate
Fed	10	30	4.1 $\pm$ 1.1	1.7 $\pm$ 0.6 ^a (42 $\pm$ 15)
	20	30	8.7 $\pm$ 0.9	N.D.
	100	90	44 $\pm$ 8	16 $\pm$ 3 ^a (38 $\pm$ 13)
Starved 1 day	100	90	82 $\pm$ 13 ^b	N.D.
Starved 3 days	10	30	2.9 $\pm$ 0.7	1.2 $\pm$ 0.2 ^a (43 $\pm$ 11)
	20	30	5.1 $\pm$ 0.9 ^b	N.D.
	100	90	29 $\pm$ 4 ^b	12 $\pm$ 4 ^a (40 $\pm$ 14)

To interpret the results of this study, it is important to know the influence of amino-oxyacetate on the rate of protein degradation during the assay for oxidative decarboxylation. For this reason hemidiaphragms from fed and from 3-day-starved rats were incubated in the presence of 0.5 mM-cycloheximide (Fulks *et al.*, 1975). To mimic the assay conditions, 0.1 mM non-radioactive 4-methyl-2-

oxopentanoate was added. After 90 min incubation total acid-soluble tyrosine amounted to 0.61 ± 0.05 and 0.74 ± 0.05 nmol/mg of muscle in incubations of hemidiaphragms from fed and 3-day-starved rats respectively ($n = 5$). These amounts did not change in the presence of 1 mM-amino-oxyacetate (97 ± 5 and $103 \pm 10\%$ of the control; $n = 5$).

To test whether the results obtained with amino-

Table 5. *Oxidative-decarboxylation rates of 4-methyl-2-oxopentanoate by soleus muscle and heart slices from fed and starved rats measured in the presence and absence of amino-oxyacetate*

Oxidative-decarboxylation rates are given as means $\pm$ S.D. for four experiments. 4-Methyl-2-oxopentanoate concentration was 0.1 mM. The relative activity in the presence of 1 mM-amino-oxyacetate (in % of the control) is given in parentheses. Abbreviation: ND, not determined. ^{a,b} Significantly different from the control at $P < 0.001$ and < 0.01 respectively. ^{c,d,e} Significantly different from the comparable incubations with fed animals at $P < 0.001$, < 0.01 and < 0.05 respectively.

	Oxidative-decarboxylation rate (nmol/min per g)			
	Soleus muscle		Heart slices	
	Control	+ Amino-oxyacetate	Control	+ Amino-oxyacetate
Fed	6.5 $\pm$ 0.6	12.0 $\pm$ 1.0 ^a (187 $\pm$ 28)	3.3 $\pm$ 0.5	6.5 $\pm$ 1.1 ^b (196 $\pm$ 29)
Starved 1 day	6.0 $\pm$ 0.9	11.5 $\pm$ 1.2 ^a (194 $\pm$ 20)	ND	ND
Starved 3 days	10.7 $\pm$ 0.4 ^c	14.0 $\pm$ 0.9 ^{b,e} (131 $\pm$ 10)	5.4 $\pm$ 0.8 ^d	7.2 $\pm$ 1.0 ^b (133 $\pm$ 8)

oxyacetate are unique to this compound or are reproducible with other transaminase inhibitors, some of the experiments were repeated in the presence and absence of L-cycloserine (Tischler *et al.*, 1982). This inhibitor appeared to be less effective than amino-oxyacetate. With 10 mM-L-cycloserine, transamination rates of 0.5 mM-leucine were decreased to 47 $\pm$ 13 and 39 $\pm$ 12% of the control, and oxidative-decarboxylation rates to 49 $\pm$ 16 and 40 $\pm$ 14%, in hemidiaphragms from fed and 3-day-starved rats respectively ($n = 3$). Just like amino-oxyacetate, L-cycloserine (10 mM) increased the oxidative-decarboxylation rate of 4-methyl-2-oxopentanoate in hemidiaphragms from fed rats (123 $\pm$ 10% of the control; $n = 5$) and not in those from 3-day-starved rats (100 $\pm$ 4%; $n = 5$).

Oxidative-decarboxylation rates were also measured at 0.1 mM-4-methyl-2-oxopentanoate in the absence and presence of 1 mM-amino-oxyacetate in rat soleus muscles and in heart slices. In both preparations starvation increased the oxidative-decarboxylation rates of the 2-oxo acid (Table 5). The differential effect of amino-oxyacetate in the two nutritional conditions was comparable with that in diaphragm (Table 3).

Discussion

An increase with starvation has been reported for oxidative-decarboxylation rates of all three branched-chain amino acids in rat quarter-diaphragms (Goldberg & Odessey, 1972) and for leucine in rat hemiatria (Tischler & Goldberg, 1980) and in slices of rat gastrocnemius muscle (Adibi *et al.*, 1974). In these studies the effect of non-radioactive amino acids originating from protein degradation was neglected, and the specific radioactivity of the oxidation precursor pool was

assumed to be approximately equal to the specific radioactivity of the medium. A starvation-induced increase in the $^{14}\text{CO}_2$ production from ^{14}C -labelled leucine was also found in the rat *in vivo* (Meikle & Klain, 1972), and in the oxidative-decarboxylation rate of leucine in perfused rat hind-quarters, when it was based on the specific radioactivity of perfusate 4-methyl-2-oxopentanoate (Hutson *et al.*, 1980).

When oxidative-decarboxylation rates are based on the specific radioactivity in the medium, oxidative-decarboxylation rates are increased in intact muscle preparations with starvation, not only for branched-chain amino acids (Table 1), but also for branched-chain 2-oxo acids (Tables 1, 3 and 5). After 1 day of starvation, primarily the transamination of leucine to 4-methyl-2-oxopentanoate was increased, since transamination of leucine increased more than oxidative decarboxylation (Tables 1 and 2) and since oxidative decarboxylation of 4-methyl-2-oxopentanoate was even decreased (Table 1). It is not well understood why transamination increased in these preparations in one direction (leucine to 4-methyl-2-oxopentanoate) and not in the other (Table 2). Cellular changes in concentrations and ratios of amino group acceptors and donors may play an important role in this phenomenon. The other results taken together suggest that starvation increases the degradation of branched-chain amino acids in rat hemidiaphragms primarily by an acceleration of the oxidative decarboxylation step.

The decrease in the production of branched-chain 2-oxo acids from non-radioactive amino acids originating from protein degradation and the inhibition of the reverse transamination of added 1- ^{14}C -labelled branched-chain 2-oxo acids towards the corresponding amino acids are probably the

main causes for the apparent increase in the oxidative decarboxylation of branched-chain 2-oxo acids in the presence of the transaminase inhibitors (Tables 3 and 5). Effects of L-cycloserine on protein degradation and synthesis and on the transport of leucine into diaphragms were ruled out by Tischler *et al.* (1982). Amino-oxyacetate also had no effect on protein degradation. Other mechanisms that may cause an increase are a direct or indirect effect on the branched-chain 2-oxo acid dehydrogenase activity and effects on the transport in or out of the cells or mitochondria of 4-methyl-2-oxopentanoate and/or leucine. Amino-oxyacetate did not influence the branched-chain 2-oxo acid dehydrogenase activity measured in homogenates and mitochondria isolated from muscles of fed and starved rats (results not shown; assay as described by Veerkamp *et al.*, 1980). This seems to argue against an effect on the enzyme activity or on the transport of 4-methyl-2-oxopentanoate into the mitochondria. In these disrupted cell preparations, however, the enzyme complex already has been activated and the mitochondrial permeability may already have been altered during preparation and preincubation. In that case the transaminase inhibitors may have no additional effect.

Since the apparent increase in the oxidative decarboxylation of branched-chain 2-oxo acids in the presence of the transaminase inhibitors was considerably larger in muscles from fed than in those from 3-day-starved rats (Tables 3 and 5), the process(es) responsible for this increase is (are) influenced by the nutritional state. Since there were no differences between muscles from fed and 3-day-starved rats in the total transamination of 4-methyl-2-oxo-[1-¹⁴C]pentanoate towards free and incorporated leucine (Table 2 and 4) and in the inhibition of that process by amino-oxyacetate, the differential effect of amino-oxyacetate cannot be related to these processes. Although a differential effect on the branched-chain 2-oxo acid dehydrogenase complex or on transport processes cannot be excluded, it seems more plausible that the transaminase inhibitors have less effect in muscles of 3-day-starved rats because of a smaller dilution of the oxidation precursor pool with 2-oxo acids derived from amino acids originating from protein degradation in these muscles. In rat heart slices this may relate to the reported decrease in protein breakdown in atrial muscle preparations (Curfman *et al.*, 1980) and perfused hearts (Crie *et al.*, 1980) from starved rats. In skeletal-muscle preparations from 80–100 g rats, protein degradation increases, however, with starvation (Fulks *et al.*, 1975; Millward *et al.*, 1976; Li & Goldberg, 1976; Li *et al.*, 1979; Goldberg *et al.*, 1980; Goodman *et al.*, 1981). In these muscles, our results may be explained by a metabolic model containing either two intra-

cellular branched-chain 2-oxo acid or two branched-chain amino acid pools, the segregation of which is more complete in muscles from 3-day-starved than from fed rats. Branched-chain 2-oxo acids arising from protein degradation may be less available to the mitochondria than are branched-chain 2-oxo acids taken up from the medium. Metabolic compartmentation was proposed for pyruvate in the isolated perfused rat heart (Peuhkurinen *et al.*, 1983). Branched-chain amino acids arising from protein degradation may also form a separate pool, which is in some way protected against transamination. The finding in the present study that amino-oxyacetate inhibits the production of free leucine from 4-methyl-2-oxopentanoate more effectively than its incorporation into protein (Table 4) suggests the existence of different functional leucine pools. Other studies on branched-chain amino acid metabolism (Nissen & Haymond, 1981; Schneible *et al.*, 1981) and on amino acid incorporation (Airhart *et al.*, 1974; O'Hara *et al.*, 1981; Rannels *et al.*, 1982; Wheatley, 1982) also gave evidence for metabolic compartmentation of leucine and other amino acids.

Our results with transaminase inhibitors in different conditions may imply that accurate oxidative-decarboxylation rates can only be based on the specific radioactivity of the mitochondrial 2-oxo acid pool. The present study indicates that starvation influences oxidation of branched-chain amino acids and 2-oxo acids in intact rat skeletal and cardiac muscle preparations considerably less than the data from Table 1 and a number of previous studies (Goldberg & Odessey, 1972; Adibi *et al.*, 1974; Hutson *et al.*, 1980; Tischler & Goldberg, 1980) suggest. This conclusion may explain the discrepancies between these studies *in vitro* and the studies *in vivo* on leucine clearance in the rat (Thomas *et al.*, 1982), dog (Nissen & Haymond, 1981) and man (Sherwin, 1978; Rennie *et al.*, 1982).

We thank Har Salden for his contribution to the preliminary experiments and Bellona Driessen for typing the manuscript.

References

- Adibi, S. A., Krzysik, B. A., Morse, E. L., Amin, P. M. & Allen, E. R. (1974) *J. Lab. Clin. Med.* **83**, 548–562
- Airhart, J., Vidrich, A. & Khairallah, E. A. (1974) *Biochem. J.* **140**, 539–548
- Crie, J. S., Sanford, C. F. & Wildenthal, K. (1980) *J. Nutr.* **110**, 22–27
- Curfman, G. D., O'Hara, D. S., Hopkins, B. E. & Smith, T. W. (1980) *Circ. Res.* **46**, 581–589
- Felig, P. (1977) *Ann. N.Y. Acad. Sci.* **301**, 56–63

- Fulks, R. M., Li, J. B. & Goldberg, A. L. (1975) *J. Biol. Chem.* **250**, 290–298
- Goldberg, A. L. & Chang, T. W. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 2301–2307
- Goldberg, A. L. & Odessey, R. (1972) *Am. J. Physiol.* **223**, 1384–1391
- Goldberg, A. L., Tischler, M., Demartino, G. & Griffin, G. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 31–36
- Goodman, M. N., McElaney, M. A. & Ruderman, N. B. (1981) *Am. J. Physiol.* **241**, E321–E327
- Hutson, S. M., Zapalowski, C., Cree, T. C. & Harper, A. E. (1980) *J. Biol. Chem.* **255**, 2418–2426
- Li, J. B. & Goldberg, A. L. (1976) *Am. J. Physiol.* **231**, 441–448
- Li, J. B., Higgins, J. E. & Jefferson, L. S. (1979) *Am. J. Physiol.* **236**, E222–E228
- Meikle, A. W. & Klain, G. J. (1972) *Am. J. Physiol.* **222**, 1246–1250
- Miller, L. L. (1962) in *Amino Acid Pools* (Holden, J. T., ed.), pp. 708–721, Elsevier, New York
- Millward, D. J., Garlick, P. J., Nnanyelugo, D. O. & Waterlow, J. C. (1976) *Biochem. J.* **156**, 185–188
- Nissen, S. & Haymond, M. W. (1981) *Am. J. Physiol.* **241**, E72–E75
- O'Hara, D. S., Curfman, G. D., Trumbull, C. G. & Smith, T. W. (1981) *J. Mol. Cell. Cardiol.* **13**, 925–940
- Peuhkurinen, K. J., Hiltunen, J. K. & Hassinen, I. E. (1983) *Biochem. J.* **210**, 193–198
- Rannels, D. E., Low, R. B., Youdale, T., Volkin, E. & Longmore, W. J. (1982) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41**, 2833–2839
- Rennie, M. J., Edwards, R. H. T., Halliday, D., Matthews, D. E., Wolman, S. L. & Millward, D. J. (1982) *Clin. Sci.* **63**, 519–523
- Schneible, P. A., Airhart, J. & Low, R. B. (1981) *J. Biol. Chem.* **256**, 4888–4894
- Sherwin, R. S. (1978) *J. Clin. Invest.* **61**, 1471–1481
- Shinnick, F. L. & Harper, A. E. (1976) *Biochim. Biophys. Acta* **437**, 477–486
- Thomas, L. K., Ittmann, M. & Cooper, C. (1982) *Biochem. J.* **204**, 399–403
- Tischler, M. E. & Goldberg, A. L. (1980) *Am. J. Physiol.* **238**, E480–E486
- Tischler, M. E., Desautels, M. & Goldberg, A. L. (1982) *J. Biol. Chem.* **257**, 1613–1621
- Veerkamp, J. H. & Wagenmakers, A. J. M. (1981) in *Metabolism and Clinical Implications of Branched-Chain Amino and Ketoacids* (Walser, M. & Williamson, J. R., eds.), pp. 163–168, Elsevier/North-Holland, New York
- Veerkamp, J. H., van Hinsbergh, V. W. M. & Cordewener, J. H. G. (1980) *Biochem. Med.* **24**, 118–129
- Waalkes, T. P. & Udenfriend, S. (1957) *J. Lab. Clin. Med.* **50**, 733–736
- Wagenmakers, A. J. M. & Veerkamp, J. H. (1982) *Biochem. Med.* **28**, 16–31
- Wheatley, D. N. (1982) *Experientia* **38**, 818–820