The effect of ketone bodies on alanine and glutamine metabolism in isolated skeletal muscle from the fasted chick

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The effects of ketone bodies on the metabolism of alanine and glutamine were studied in isolated extensor digitorum communis (EDC) muscles from 24 h-fasted chicks. (1) Acetoacetate and DL- β -hydroxybutyrate (4 mM) markedly inhibit branched-chain amino acid (BCAA) transamination and alanine formation. (2) Ketone bodies (1 and 4 mM) increase the intracellular concentration and release of glutamate and glutamine, suggesting that inhibition of BCAA transamination does not limit intracellular availability of glutamate for alanine synthesis. (3) Ketone bodies (1 and 4 mM) do not affect glucose uptake by muscles, but decrease the rate of glycolysis as well as the intracellular concentration and release of pyruvate in muscles. (4) Addition of 12 mM-glucose increases the formation of alanine in muscles incubated in the absence of ketone bodies, but has no effect in muscles incubated in the presence of 4 mM ketone bodies. (5) Addition of 5 mM-pyruvate to the media prevents the inhibiting effect of ketone bodies on BCAA transamination and alanine synthesis. These results suggest that ketone bodies decrease alanine synthesis by limiting the intracellular availability of pyruvate, owing to inhibition of glycolysis, and inhibit BCAA transamination by decreasing the intracellular concentration of amino-group acceptors such as pyruvate in EDC muscles from fasted chicks.

INTRODUCTION

Alanine and glutamine are the predominant amino acids released by skeletal muscles of rats [1–3], humans [4] and chicks [5] and play an important role in the inter-organ metabolism of nitrogen and carbon during fasting and acidosis [4,6,7]. It is well documented that branched-chain amino acids (BCAA) provide amino groups for synthesis *de novo* of alanine and glutamine in rat skeletal muscles [8–14]. However, it remains unclear whether the major source of carbon for alanine synthesis is glycolysis [8,12,13] or amino acids which generate C_4 intermediates of the tricarboxylic acid cycle [2,3,14].

Ketone bodies have been implicated to play a role in alanine and glutamine metabolism in skeletal muscle [15–18]. The infusion of DL- β -hydroxybutyrate has been found to decrease plasma concentrations of alanine in fasted man [19] and dogs [20], suggesting decreased production and release of alanine from skeletal muscle [19,20]. Similarly, studies *in vitro* have demonstrated that acetoacetate and DL- β -hydroxybutyrate suppress the formation of alanine [15,17,18] and glutamine [15] in skeletal muscles from fasted rats. In addition, ketone bodies have been reported to decrease alanine release from cultured rat skeletal-muscle cells [21] and from perfused rat hindquarters [22]. The mechanisms by which ketone bodies regulate amino acid metabolism have not been elucidated.

It has been suggested that ketone bodies decrease alanine formation in fasted-rat diaphragms *in vitro* [15] and in fasted dogs *in vivo* [20] by decreasing the tissue concentration of pyruvate owing to inhibition of glycolysis [15,20]. But neither the rate of glycolysis nor the intracellular concentration of pyruvate was measured in

those studies. The possible role of glycolysis has been questioned, since ketone bodies have been found to decrease alanine release from cultured rat skeletal-muscle cells [21] and perfused rat hindquarters [22] without influencing either the rate of glycolysis or intracellular pyruvate concentration in these preparations [21,22]. Thus, since ketone bodies have been shown to inhibit BCAA metabolism in perfused hindquarters [23] and incubated diaphragms [24] of rats and to decrease intracellular glutamate concentration in cultured rat skeletal-muscle cells [21], several researchers have suggested that ketone bodies decrease alanine synthesis in skeletal muscle by directly inhibiting BCAA transamination [17,21]. This suggestion appears to be supported by our recent findings that ketone bodies markedly inhibit BCAA transamination in skeletal muscle from fasted chicks [25]. The present study was therefore conducted to test this hypothesis in an attempt to determine how ketone bodies regulate alanine and glutamine metabolism in skeletal muscles from fasted chicks.

MATERIALS AND METHODS

Chemicals

L-[1- 14 C]Leucine, L-[1- 14 C]valine and L-[U- 14 C]alanine were purchased from ICN Radiochemicals, Montreal, Que., Canada. D-[5- 3 H]Glucose and 3-*O*-[Me- 14 C]-methylglucose were purchased from American Radio-labeled Chemicals, St. Louis, MO, U.S.A. L-Lactate dehydrogenase and all other chemicals used were purchased from Sigma Chemical Co., St. Louis MO, U.S.A.

Abbreviations used: EDC, extensor digitorum communis; BCAA, branched-chain amino acid.

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Animals

Day-old male broiler chicks (Hubbard strain, *Gallus domesticus*) were obtained from a local hatchery and allowed free access to food and water. At 9 days of age, chicks weighing 115–125 g were fasted for 24 h before use.

Muscle preparations and incubation

Individual fasted chicks were anaesthetized with halothane. The extensor digitorum communis (EDC) muscles (15.8 ± 0.2 mg; mean $\pm$ S.E.M., $n = 20$) were dissected and placed in 3.5 ml of Krebs–Ringer bicarbonate buffer saturated with O_2/CO_2 (19:1) [25]. The buffer also contained insulin (0.01 unit/ml) and 2 mM-Hepes (basal medium). Glucose and amino acids were present in or absent from the incubation media depending on the experimental design. In all experiments, one EDC muscle was used for the control while the contralateral muscle was used for the treatment. The muscles were pre-incubated for 30 min and then incubated for 2 h in fresh media at 37 °C [25], with additions as indicated in Tables 1–5.

Measurement of the release of glutamate, glutamine and alanine and intracellular concentrations of amino acids

In experiments designed to measure the amounts of alanine, glutamate, glutamine and tyrosine in the muscle proteins, in the intracellular free pool and released to the incubation media, EDC muscles were incubated in the basal medium containing 12 mM-glucose as described above. In experiments designed to determine the effect of pyruvate and ketone bodies on amino acid metabolism, the basal media contained 12 mM-glucose, 0.5 mM-leucine, 0.5 mM-valine, and all other amino acids at plasma concentrations [26], except for alanine, glutamine, glutamate, tyrosine and histidine, which were absent (medium B). Medium B containing either 0 or 12 mM-glucose was used in studies of the effect of glucose on amino acid metabolism. At the end of the final incubation period, muscles were placed in ice-cold 2% (w/v) trichloroacetic acid. The incubation media and the muscles were stored at -70 °C, and amino acids were analysed within 2 weeks by h.p.l.c. as described previously [25,27].

Measurement of BCAA transamination

EDC muscles were incubated in medium B containing 0.5 mM-L-[1- ^{14}C]leucine (400 d.p.m./nmol) or 0.5 mM-L-[1- ^{14}C]valine (400 d.p.m./nmol) as described above. At the end of the 2 h final incubation, $^{14}CO_2$ was collected in Hyamine hydroxide [11,12,25], and the net transamination rates of leucine and valine were calculated as described previously [25,28].

Measurement of alanine oxidation

EDC muscles were incubated in medium B containing 1.0 mM-L-[U- ^{14}C]alanine (106 d.p.m./nmol) as described above. At the end of the final incubation, muscles were placed in 1 M-HClO₄ and $^{14}CO_2$ was collected from the incubation media in Hyamine hydroxide [25]. Neutralized muscle extracts were passed through a column (1.0 cm $\times$ 13 cm) containing Dowex 1-10X resin (formate form) to separate alanine from the other metabolizable amino acids and tricarboxylic-acid-cycle intermediates [29–31]. The fractions collected contained alanine,

glutamine and asparagine, but not glutamate, aspartate, lactate or pyruvate, as verified by h.p.l.c. for amino acids [25,27] and by an enzymic fluorimetric assay for lactate and pyruvate [32]. To convert glutamine and asparagine into glutamate and aspartate respectively, each fraction was incubated with 0.5 ml of 6 M-HCl under N_2 at 110 °C for 10 h. The neutralized solution was passed through a Dowex 1-10X (formate form) column as described above. A 1 ml portion of the fraction containing alanine was counted for radioactivity [25], and 0.2 ml was assayed for alanine by h.p.l.c. [25,27]. The specific radioactivity of intracellular L-[U- ^{14}C]alanine was calculated after correction for that of alanine in the extracellular space [25], and was used to calculate the rate of alanine oxidation.

Measurement of glucose uptake and glycolysis

Glucose uptake was measured in EDC muscles incubated in medium B containing 5 mM-3-*O*-[Me- ^{14}C]-methylglucose (38 d.p.m./nmol) [33–35]. At the end of the 2 h incubation, each EDC muscle was well rinsed with non-radioactive incubation media, solubilized in 0.5 ml of Soluene and counted for radioactivity [25]. The intracellular radioactivity of 3-*O*-[Me- ^{14}C]methylglucose was calculated after correction for that in the extracellular space [25].

Glycolysis was measured in EDC muscles incubated in medium B containing 12 mM-D-[5- 3H]glucose (50 d.p.m./nmol) [8]. At the end of the 2 h incubation, 3H_2O accumulated in the incubation media was separated from D-[5- 3H]glucose, and the rate of glycolysis was calculated as described by Chang & Goldberg [8].

Measurement of the intracellular concentration and release of pyruvate

EDC muscles were incubated in medium B as described above. At the end of the 2 h final incubation, each muscle was placed in 0.85 ml of ice-cold 1.0 M-HClO₄ and the incubation medium was acidified with 0.2 ml of 1.0 M-HClO₄. Neutralized muscle extracts and media were analysed immediately for pyruvate [32]. The intracellular concentration of pyruvate was calculated after correction for pyruvate in the extracellular space [25].

Statistical analysis of results

All results, given as means $\pm$ S.E.M., were analysed by comparing the mean value of the treated muscles with that of the contralateral control muscles by the paired *t* test as described by Steel & Torrie [36].

RESULTS

Effects of ketone bodies, glucose and pyruvate on the intracellular concentration and release of glutamate, glutamine and alanine

A relatively large amount of alanine and glutamine and a relatively small amount of glutamate are released from fasted-chick EDC muscles during a 2 h final incubation period (Table 1). The intracellular concentrations of these amino acids remain constant throughout the final incubation (results not shown). The alanine/tyrosine molar ratio in the incubation media and in the intracellular free amino acid pool is 10.3 ± 0.4 ($n = 10$) and 13.3 ± 0.7 ($n = 10$) respectively, which is much higher ($P < 0.001$) than that in EDC muscle proteins (5.0 ± 0.2 ; $n = 10$). The ratio (glutamate + glutamine)/tyrosine in

Table 1. Effect of ketone bodies, glucose and pyruvate on the intracellular concentrations and the release of glutamate, glutamine and alanine in EDC muscles from 24 h-fasted chicks

EDC muscles from fasted chicks were incubated in media containing no glucose (None) or glucose (12 mM) as described in the text. Acetoacetate (4 mM), DL- β -hydroxybutyrate (4 mM) or pyruvate (5 mM) was added to the incubation media of the 'treatment' muscles as indicated. Results are given as means $\pm$ S.E.M. for ten observations per treatment and statistically analysed by the paired *t* test. Treatment means marked with * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$) are different from the control means obtained from the contralateral muscles.

Additions to incubation medium	Intracellular concn. (nmol/mg of tissue)			Release (nmol/2 h per mg of tissue)		
	Glutamate	Glutamine	Alanine	Glutamate	Glutamine	Alanine
None	1.56 $\pm$ 0.09	2.49 $\pm$ 0.19	1.13 $\pm$ 0.05	0.46 $\pm$ 0.03	2.44 $\pm$ 0.17	1.18 $\pm$ 0.08
Glucose	1.20 $\pm$ 0.06**	2.06 $\pm$ 0.15**	2.19 $\pm$ 0.18***	0.36 $\pm$ 0.02*	2.12 $\pm$ 0.18**	2.10 $\pm$ 0.19***
None	1.53 $\pm$ 0.12	2.43 $\pm$ 0.24	1.19 $\pm$ 0.08	0.44 $\pm$ 0.04	2.36 $\pm$ 0.19	1.26 $\pm$ 0.17
Glucose + acetoacetate	2.87 $\pm$ 0.14***	2.51 $\pm$ 0.18	1.32 $\pm$ 0.12	0.55 $\pm$ 0.03**	2.40 $\pm$ 0.23	1.44 $\pm$ 0.19
None	1.48 $\pm$ 0.13	2.27 $\pm$ 0.19	1.25 $\pm$ 0.15	0.40 $\pm$ 0.02	2.34 $\pm$ 0.09	1.21 $\pm$ 0.16
Glucose + DL- β -hydroxybutyrate	2.06 $\pm$ 0.16***	2.45 $\pm$ 0.17	1.43 $\pm$ 0.17	0.52 $\pm$ 0.03**	2.51 $\pm$ 0.15	1.39 $\pm$ 0.20
Glucose	1.35 $\pm$ 0.05	2.14 $\pm$ 0.12	2.32 $\pm$ 0.16	0.39 $\pm$ 0.04	1.98 $\pm$ 0.11	2.05 $\pm$ 0.09
Glucose + acetoacetate	3.07 $\pm$ 0.11***	2.59 $\pm$ 0.20**	1.28 $\pm$ 0.11***	0.55 $\pm$ 0.05**	2.41 $\pm$ 0.09**	1.26 $\pm$ 0.05***
Glucose	1.23 $\pm$ 0.04	2.18 $\pm$ 0.10	2.16 $\pm$ 0.08	0.36 $\pm$ 0.04	2.01 $\pm$ 0.17	1.97 $\pm$ 0.12
Glucose + DL- β -hydroxybutyrate	1.91 $\pm$ 0.06***	2.62 $\pm$ 0.06**	1.51 $\pm$ 0.11***	0.51 $\pm$ 0.04**	2.53 $\pm$ 0.16***	1.44 $\pm$ 0.05***
Glucose	1.38 $\pm$ 0.07	2.38 $\pm$ 0.15	2.13 $\pm$ 0.18	0.38 $\pm$ 0.02	2.13 $\pm$ 0.12	1.98 $\pm$ 0.13
Glucose + pyruvate	1.06 $\pm$ 0.04***	1.90 $\pm$ 0.18**	2.84 $\pm$ 0.22***	0.29 $\pm$ 0.02**	1.82 $\pm$ 0.16**	2.71 $\pm$ 0.24***
Glucose + pyruvate	1.04 $\pm$ 0.06***	1.81 $\pm$ 0.14	2.79 $\pm$ 0.12	0.33 $\pm$ 0.02	1.78 $\pm$ 0.18	2.75 $\pm$ 0.10
Glucose + pyruvate + acetoacetate	1.76 $\pm$ 0.05***	1.93 $\pm$ 0.12	2.68 $\pm$ 0.09	0.50 $\pm$ 0.03***	1.86 $\pm$ 0.14	2.89 $\pm$ 0.17
Glucose + pyruvate	1.11 $\pm$ 0.06	1.88 $\pm$ 0.12	2.73 $\pm$ 0.13	0.31 $\pm$ 0.03	1.87 $\pm$ 0.14	2.86 $\pm$ 0.14
Glucose + pyruvate + DL- β -hydroxybutyrate	1.62 $\pm$ 0.08***	2.01 $\pm$ 0.17	2.77 $\pm$ 0.17	0.42 $\pm$ 0.04**	1.75 $\pm$ 0.15	2.62 $\pm$ 0.20

Table 2. Effect of ketone bodies on the intracellular concentrations of aspartate and BCAA in EDC muscles from 24 h-fasted chicks

EDC muscles from fasted chicks were incubated as described in the text. Glucose (12 mM) was present in the media. Acetoacetate (4 mM), DL- β -hydroxybutyrate (4 mM) or pyruvate (5 mM) was added to the incubation media of the 'treatment' muscles as indicated. Results are given as means $\pm$ S.E.M. for ten observations per treatment and statistically analysed by the paired *t* test. Treatment means marked with ** ($P < 0.01$) and *** ($P < 0.001$) are different from the control means obtained from the contralateral muscles.

Additions to incubation medium	Concn. (nmol/mg of tissue)			
	Aspartate	Valine	Isoleucine	Leucine
None	1.12 $\pm$ 0.04	0.42 $\pm$ 0.01	0.19 $\pm$ 0.01	0.38 $\pm$ 0.01
Acetoacetate	0.69 $\pm$ 0.02***	0.61 $\pm$ 0.02***	0.28 $\pm$ 0.01***	0.54 $\pm$ 0.02***
None	1.08 $\pm$ 0.06	0.44 $\pm$ 0.01	0.21 $\pm$ 0.01	0.41 $\pm$ 0.01
DL- β -Hydroxybutyrate	0.78 $\pm$ 0.05**	0.55 $\pm$ 0.02***	0.27 $\pm$ 0.02***	0.52 $\pm$ 0.03***
Pyruvate	0.95 $\pm$ 0.02	0.45 $\pm$ 0.04	0.20 $\pm$ 0.02	0.39 $\pm$ 0.03
Pyruvate + acetoacetate	0.94 $\pm$ 0.06	0.47 $\pm$ 0.06	0.21 $\pm$ 0.03	0.40 $\pm$ 0.02
Pyruvate	0.99 $\pm$ 0.04	0.43 $\pm$ 0.05	0.20 $\pm$ 0.02	0.40 $\pm$ 0.05
Pyruvate + DL- β -hydroxybutyrate	1.03 $\pm$ 0.04	0.46 $\pm$ 0.04	0.22 $\pm$ 0.03	0.41 $\pm$ 0.04

the incubation media and in the intracellular free pool is 12.2 $\pm$ 0.8 ($n = 10$) and 22.7 $\pm$ 1.0 ($n = 10$) respectively, which is also much higher ($P < 0.001$) than that in EDC muscle proteins (6.1 $\pm$ 0.3; $n = 10$).

Glucose (12 mM) decreased ($P < 0.05$) the intracellular concentration and release of glutamate and glutamine, but increased ($P < 0.001$) the intracellular concentration

and release of alanine in EDC muscles incubated in the absence of ketone bodies (Table 1). In the presence of 4 mM ketone bodies, 12 mM-glucose had no effect on the intracellular concentration and release of glutamine and alanine, but increased ($P < 0.01$) the concentration and release of glutamate (Table 1).

Ketone bodies (4 mM) markedly increased ($P < 0.01$)

Table 3. Effect of pyruvate and ketone bodies on transamination of leucine and valine in EDC muscles from 24 h-fasted chicks

EDC muscles from fasted chicks were incubated with L-[1-¹⁴C]leucine or L-[1-¹⁴C]valine for 2 h as described in the text. Glucose (12 mM) was present in the incubation media. Pyruvate (5 mM), acetoacetate (4 mM) or DL- β -hydroxybutyrate (4 mM) was added to the incubation media of the 'treatment' muscles as indicated. Results are given as means $\pm$ S.E.M. for 12 observations per treatment and statistically analysed by the paired *t* test. Treatment means marked with *** ($P < 0.001$) are different from the control means obtained from the contralateral muscles.

Additions to incubation medium	Net rate of transamination (nmol/2 h per mg of tissue)	
	Leucine	Valine
None	1.46 $\pm$ 0.08	0.80 $\pm$ 0.09
Acetoacetate	0.66 $\pm$ 0.04***	0.38 $\pm$ 0.06***
None	1.40 $\pm$ 0.14	0.76 $\pm$ 0.07
DL- β -Hydroxybutyrate	0.78 $\pm$ 0.06***	0.44 $\pm$ 0.04***
Pyruvate	1.34 $\pm$ 0.11	0.72 $\pm$ 0.04
Pyruvate + acetoacetate	1.30 $\pm$ 0.08	0.68 $\pm$ 0.04
Pyruvate	1.42 $\pm$ 0.10	0.80 $\pm$ 0.08
Pyruvate + DL- β -hydroxybutyrate	1.36 $\pm$ 0.07	0.82 $\pm$ 0.10

the intracellular concentration and release of glutamate and glutamine, but decreased ($P < 0.001$) the intracellular concentration and release of alanine in EDC muscles incubated with 12 mM-glucose (Table 1). Similar results were obtained with 1 mM-acetoacetate, and 1 mM-DL- β -hydroxybutyrate slightly increased ($P < 0.05$) the intracellular concentration and release of glutamate, glutamine and alanine (results not shown). However, ketone bodies (4 mM) did not influence ($P > 0.05$) the intracellular concentration and release of glutamine and alanine in EDC muscles incubated with 5 mM-pyruvate (Table 1).

Effect of ketone bodies on intracellular concentrations of aspartate and BCAAs

Acetoacetate (4 mM) and DL- β -hydroxybutyrate (4 mM) decreased ($P < 0.001$) the intracellular concentration of aspartate by 38 % and 28 %, but increased ($P < 0.001$) the intracellular concentrations of valine, isoleucine and leucine by 42–47 % and 25–27 % respectively, in EDC muscles incubated with 12 mM-glucose (Table 2). However, 4 mM ketone bodies had no effect ($P > 0.05$) on the concentration of these amino acids in EDC muscles incubated in the presence of 5 mM-pyruvate (Table 2). Similar results were obtained in the presence of 1 mM ketone bodies (results not shown). In addition, 1 and 4 mM ketone bodies did not influence ($P > 0.05$) the intracellular concentration of asparagine and amino acids not metabolized in fasted-chick EDC muscles (results not shown).

Effect of ketone bodies and pyruvate on BCAA transamination

Ketone bodies (4 mM) inhibited ($P < 0.001$) the net transamination rates of leucine and valine by 40–52 % in EDC muscles incubated in the absence of pyruvate (Table 3). Addition of 5 mM-pyruvate to the incubation media prevented the inhibitory effect of ketone bodies on BCAA transamination (Table 3).

Effect of ketone bodies on glucose uptake, glycolysis and the intracellular concentration and release of pyruvate

Acetoacetate and DL- β -hydroxybutyrate (1 and 4 mM) did not influence ($P > 0.05$) 3-*O*-[Me-¹⁴C]methylglucose uptake by EDC muscles (Table 4). However, acetoacetate (4 mM) and DL- β -hydroxybutyrate (4 mM) decreased ($P < 0.001$) the rate of glycolysis by 41 % and 34 %, the intracellular concentration of pyruvate by 61 % and 50 %, and the release of pyruvate by 64 % and 52 %, respectively, in EDC muscles incubated with 12 mM-glucose (Table 4). Ketone bodies at 1 mM also decreased ($P < 0.01$) the rate of glycolysis and the intracellular concentration and release of pyruvate (results not shown).

Effect of ketone bodies on alanine oxidation

Acetoacetate (4 mM) and DL- β -hydroxybutyrate (4 mM) increased ($P < 0.01$) the specific radioactivity of

Table 4. Effect of ketone bodies on 3-*O*-methylglucose uptake, glycolysis, and the intracellular concentration and release of pyruvate in EDC muscles from 24 h-fasted chicks

EDC muscles were incubated with 5 mM-3-*O*-[Me-¹⁴C]methylglucose and 12 mM-glucose to measure glucose uptake, or with 12 mM-D-[5-³H]glucose to measure glycolysis, as described in the text. Acetoacetate (4 mM) or DL- β -hydroxybutyrate (4 mM) was added to the incubation media of the 'treatment' muscles as indicated. The results are given as means $\pm$ S.E.M. for six observations per treatment and statistically analysed by the paired *t* test. Treatment means marked with *** ($P < 0.001$) are different from the control means obtained from the contralateral muscles.

Additions to incubation medium	Uptake of 3- <i>O</i> -[Me- ¹⁴ C]-methylglucose (d.p.m./2 h per mg of tissue)	Rate of glycolysis (nmol of glucose/2 h per mg of tissue)	Pyruvate	
			Intracellular concn. (nmol/mg of tissue)	Release (nmol/2 h per mg of tissue)
None	113.4 $\pm$ 3.3	23.22 $\pm$ 0.92	0.31 $\pm$ 0.03	5.49 $\pm$ 0.27
Acetoacetate	111.6 $\pm$ 1.5	13.68 $\pm$ 0.80***	0.12 $\pm$ 0.01***	1.95 $\pm$ 0.16***
None	110.8 $\pm$ 1.9	23.36 $\pm$ 1.10	0.28 $\pm$ 0.02	5.35 $\pm$ 0.34
DL- β -Hydroxybutyrate	114.2 $\pm$ 2.8	15.40 $\pm$ 0.82***	0.14 $\pm$ 0.01***	2.55 $\pm$ 0.32***

Table 5. Effect of ketone bodies on alanine oxidation in EDC muscles from 24 h-fasted chicks

EDC muscles from fasted chicks were incubated with 1.0 mM-L-[U-¹⁴C]alanine for 2 h as described in the text. Glucose (12 mM) was present in the incubation medium. Acetoacetate (4 mM) or DL- β -hydroxybutyrate (4 mM) was added to the incubation media of the treatment muscles as indicated. The results are given as means $\pm$ S.E.M. for six observations per treatment and statistically analysed by the paired *t* test. Treatment means marked with **(*P* < 0.01) and ***(*P* < 0.001) are different from the control means obtained from the contralateral muscles.

Additions to incubation medium	Sp. radio-activity of intracellular alanine (d.p.m./nmol)	Alanine oxidation (nmol/2 h per mg of tissue)
None	55.4 $\pm$ 3.1	1.22 $\pm$ 0.06
Acetoacetate	69.0 $\pm$ 2.5**	0.63 $\pm$ 0.07***
None	63.2 $\pm$ 4.1	1.26 $\pm$ 0.13
DL- β -Hydroxybutyrate	75.3 $\pm$ 3.8**	0.59 $\pm$ 0.06***

intracellular L-[U-¹⁴C]alanine by 25 % and 19 % respectively, and decreased (*P* < 0.001) the rate of alanine oxidation by 48 % and 53 % respectively, in chick EDC muscles (Table 5). Ketone bodies at 1 mM also inhibited (*P* < 0.001) the rate of alanine oxidation in EDC muscles incubated in the presence of 12 mM-glucose (results not shown).

DISCUSSION

The present study demonstrates that alanine and glutamine are released by chick skeletal muscle to a much greater extent than would be expected from their abundance in muscle proteins. Although alanine oxidation is inhibited by ketone bodies (Table 5), which has not been reported in other species, the intracellular concentration and the release of alanine in fasted-chick EDC muscles are markedly decreased by 1 mM- and 4 mM-acetoacetate and by 4 mM-DL- β -hydroxybutyrate (Table 1). Our results indicate that ketone bodies suppress alanine synthesis in chick skeletal muscle, as previously shown in rat skeletal muscle [15,17,18].

It is unlikely that inhibition of BCAA transamination by ketone bodies limits intracellular glutamate availability for alanine synthesis, since the intracellular glutamate concentration is markedly increased by ketone bodies in fasted-chick EDC muscles (Table 1). Thus ketone bodies probably decrease alanine synthesis in skeletal muscles by decreasing intracellular pyruvate concentration, which may also result in decreased BCAA transamination owing to decreased regeneration of 2-oxoglutarate. This conclusion is supported by the following observations. (1) Ketone bodies have no effect on glucose uptake, but markedly inhibit the rate of glycolysis in fasted-chick EDC muscles, resulting in a dramatic decrease in the intracellular concentration and release of pyruvate (Table 4). (2) Glucose increases alanine formation in EDC muscles incubated in the absence of ketone bodies, but has no effect on alanine

formation in EDC muscles incubated in the presence of 4 mM ketone bodies (Table 1). (3) The addition of pyruvate, which does not affect BCAA transamination in fasted-chick EDC muscles incubated in the absence of ketone bodies [28], prevents the inhibitory effect of ketone bodies on BCAA transamination in fasted-chick EDC muscles (Table 3) and on alanine formation in both fasted-chick EDC muscles (Table 1) and fasted-rat diaphragms [15].

The effect of ketone bodies on glutamine metabolism is not well documented in skeletal muscle. In the present study, it is found that ketone bodies markedly increase both the intracellular concentration and the release of glutamine from fasted-chick EDC muscles (Table 1). Our results are in sharp contrast with those of Palaiologos & Felig [15], who reported that ketone bodies decrease glutamine release from fasted-rat hemidiaphragms. An increase in the intracellular concentration and in the release of glutamine by ketone bodies may be attributed to an increased intracellular glutamate concentration (Table 1), which has been shown to increase glutamine synthetase activity in rat skeletal-muscle cells [37]. It is also noteworthy that an increase in both glutamine synthetase activity and glutamine release has been reported in skeletal muscles of diabetic ketoacidotic rats, in which plasma concentrations of ketone bodies are elevated [38,39].

Increased release of glutamine at the expense of alanine may be of physiological significance to animals with high plasma concentrations of ketone bodies. Decreased release of alanine from skeletal muscles contributes to decreased plasma concentrations of alanine [4,19], which may result in decreased hepatic protein synthesis during prolonged starvation [40], since alanine has been implicated to be rate-limiting for hepatic protein synthesis [40]. On the other hand, an increase in the release of glutamine from skeletal muscle provides more glutamine for removal by the kidneys, where it plays a key role in the excretion of H⁺ ions during acidosis [41]. Also, an increase in the release of glutamine from skeletal muscle may help to inhibit lipolysis and hence hepatic ketogenesis during fasting [42,43], thereby preventing a further increase in plasma ketone bodies.

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REFERENCES

1. Ruderman, N. B. & Berger, M. (1974) *J. Biol. Chem.* **249**, 5500–5506
2. Ozand, P. T., Tildon, J. T., Wapnir, R. A. & Cornblath, M. (1973) *Biochem. Biophys. Res. Commun.* **53**, 251–257
3. Garber, A. J., Karl, I. E. & Kipnis, D. M. (1976) *J. Biol. Chem.* **251**, 826–835
4. Felig, P. (1975) *Annu. Rev. Biochem.* **44**, 933–955
5. Tinker, D. A., Brosnan, J. T. & Herzberg, G. R. (1986) *Biochem. J.* **240**, 829–836
6. Smith, R. J. (1986) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **45**, 2172–2176
7. Goldstein, L. (1986) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **45**, 2176–2179

8. Chang, T. W. & Goldberg, A. L. (1978) *J. Biol. Chem.* **253**, 3677–3684
9. Chang, T. W. & Goldberg, A. L. (1978) *J. Biol. Chem.* **253**, 3685–3695
10. Snell, K. (1980) *Biochem. Soc. Trans.* **8**, 205–213
11. Snell, K. & Duff, D. A. (1985) *Biochem. J.* **225**, 737–743
12. Caldecourt, M. A., Cox, D. J., Sugden, M. C. & Palmer, T. N. (1985) *Biochem. J.* **231**, 801–804
13. Palmer, T. N., Caldecourt, M. A., Snell, K. & Sugden, M. C. (1985) *Biosci. Rep.* **5**, 1015–1020
14. Goldstein, L. & Newsholme, E. A. (1976) *Biochem. J.* **154**, 555–558
15. Palaiologos, G. & Felig, P. (1976) *Biochem. J.* **154**, 709–716
16. Robinson, A. M. & Williamson, D. H. (1980) *Physiol. Rev.* **60**, 143–187
17. Palmer, T. N., Caldecourt, M. A., Warner, J. P. & Sugden, M. C. (1985) *Biochem. Int.* **11**, 407–413
18. Snell, K. & Duff, D. A. (1982) in *Cell Function and Differentiation, Part C* (Akoyunoglou, G., ed.), pp. 279–291, Alan R. Liss, New York
19. Sherwin, R. S., Hendler, R. G. & Felig, P. (1975) *J. Clin. Invest.* **55**, 1382–1390
20. Umpleby, A. M., Chubb, D., Boroujerdi, M. A. & Sonksen, P. H. (1988) *Clin. Sci.* **74**, 41–48
21. Smith, R. J. & Aoki, T. T. (1981) in *Metabolism and Clinical Implications of Branched Chain Amino and Ketoacids* (Walsher, M. & Williamson, J. R., eds.), pp. 271–276, Elsevier/North-Holland, New York
22. Berger, M., Kemmer, F. W., Goodman, M. N., Zimmermann-Telschow, H. & Ruderman, N. B. (1978) in *Biochemical and Clinical Aspects of Ketone Body Metabolism* (Soling, H. D. & Seufert, C. D., eds.), pp. 193–203, Thieme, Stuttgart
23. Zapalowski, G., Hutson, S. M. & Harper, A. E. (1981) in *Metabolism and Clinical Implications of Branched Chain Amino and Ketoacids* (Walsher, M. & Williamson, J. R., eds.), pp. 239–244, Elsevier/North-Holland, New York
24. Buse, M. G., Biggers, J. F., Friderici, K. H. & Buse, J. F. (1972) *J. Biol. Chem.* **247**, 8085–8096
25. Wu, G. & Thompson, J. R. (1987) *Int. J. Biochem.* **19**, 937–943
26. Maruyama, K., Sunde, M. L. & Harper, A. E. (1976) *Poult. Sci.* **55**, 1615–1626
27. Jones, B. N. & Gilligan, J. P. (1983) *J. Chromatogr.* **266**, 471–482
28. Wu, G. & Thompson, J. R. (1988) *Int. J. Biochem.* **20**, 521–526
29. Busch, H., Hurlbert, R. B. & Potter, V. R. (1952) *J. Biol. Chem.* **196**, 717–727
30. LaNoue, K., Nicklas, W. J. & Williamson, J. R. (1970) *J. Biol. Chem.* **245**, 102–111
31. Lee, S. & Davis, E. J. (1979) *J. Biol. Chem.* **254**, 420–430
32. Olsen, C. (1971) *Clin. Chim. Acta* **33**, 293–300
33. Kohn, P. G. & Clausen, T. (1971) *Biochim. Biophys. Acta* **225**, 277–290
34. Clausen, T. & Flatman, J. A. (1987) *Am. J. Physiol.* **252**, E492–E499
35. Ploug, T., Galbo, H., Vinten, J., Jorgensen, M. & Richter, E. A. (1987) *Am. J. Physiol.* **253**, E12–E20
36. Steel, R. G. D. & Torrie, J. H. (1980) *Principles and Procedures of Statistics*, McGraw-Hill, New York
37. Smith, R. J., Larson, S., Stred, S. & Durschlag, R. P. (1984) *J. Cell. Physiol.* **120**, 197–203
38. Goldstein, L., Perlman, D. F., McLaughlin, P. M., King, P. A. & Cha, C.-J. (1983) *Biochem. J.* **214**, 757–767
39. King, P. A., Golstein, L. & Newsholme, E. A. (1983) *Biochem. J.* **216**, 523–525
40. Perez-Sala, D., Parrilla, R. & Ayuso, M. S. (1987) *Biochem. J.* **241**, 491–498
41. Goldstein, L. (1976) *Int. Rev. Physiol.* **2**, 283–316
42. Cersosimo, E., Williams, P., Hoxworth, B., Lacy, W. & Abumrad, N. (1986) *Am. J. Physiol.* **250**, E248–E252
43. Cersosimo, E., Williams, P. E., Radosevich, P. M., Hoxworth, B. T., Lacy, W. W. & Abumrad, N. N. (1986). *Am. J. Physiol.* **250**, E622–E628