Activities of branched-chain amino acid aminotransferase and branched-chain 2-oxo acid dehydrogenase complex in tissues of maternal and fetal sheep

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Branched-chain amino acid aminotransferase (BCAAT) and branched-chain 2-oxo acid dehydrogenase (BCODH) activities were determined in sheep maternal and fetal liver, kidney, skeletal muscle, adipose and placenta in the fed and 5-day-starved states at 125 days gestation (term is 147 days). BCAAT activities were quite low in maternal skeletal muscle, low in fetal and maternal liver, high in fetal muscle, and very high in placenta. No significant changes occurred with maternal starvation. The high BCAAT activity of the placenta may provide the essential branched-chain 2-oxo acids for the fetus. BCODH activity was highest in liver and kidney of both the mother and fetus; it was largely in the active (dephosphorylated) state in maternal liver and kidney, and in fetal kidney, and about 50% active in fetal liver. The enzyme was largely inactive in fetal muscle, and about 50% active in maternal muscle. Although starvation had little effect on the activity of BCODH in fetal tissues, a significant decrease in activity was observed in maternal tissues, thereby potentially sparing branched-chain amino acids or the corresponding 2-oxo acids for maintenance of the fetus during compromised maternal nutrition.

INTRODUCTION

Branched-chain amino acid (BCAA) metabolism involves reversible transamination followed by oxidation. The committed step in the oxidative process is catalysed by branched-chain 2-oxo acid dehydrogenase (BCODH; EC 1.2.4.4, no EC number for the acyltransferase, and EC 1.8.1.4), a mitochondrial multienzyme complex which is regulated by covalent modification. The complex is inactivated by phosphorylation and activated by dephosphorylation. It is generally accepted that muscle is the main site of transamination in the rodent. The low activity of branched-chain amino acid aminotransferase (BCAAT; EC 2.6.1.42) in rodent liver, compared with skeletal muscle, has led to the suggestion of inter-organ transport of the branched-chain 2-oxo acids to the liver for oxidation [1].

Sheep have been a commonly used model for studies of metabolism during pregnancy. Amino acids comprise as much as 20–25% of the oxidative substrate requirement for the fetal lamb [2]. In addition, it is probable that the fetus depends on amino acids to an even greater extent during maternal starvation, as evidenced by increased fetal urea excretion [3]. Several lines of evidence suggest that the BCAAs (leucine, valine and isoleucine) are a particularly important group of amino acids in sheep fetal metabolism. First, they account for about one-quarter of the total amino acid uptake by the fetal lamb [4]. Secondly, uterine uptake of the BCAAs exceeds umbilical uptake in the sheep, suggesting that they are metabolized in the placenta [4]. However, the mode of metabolism of the BCAAs in this organ has not been elucidated. Finally, the blood concentrations of the

BCAAs exhibit unique changes during maternal starvation of the pregnant ewe. In the fetus, although most amino acids remain constant, the BCAAs increase. In the mother, whereas most amino acids decrease (especially gluconeogenic amino acids), the BCAAs increase [5].

As we recognized the importance of BCAAs in a number of species, the present study was undertaken to characterize BCAA metabolism in the pregnant ewe further by determining tissue activities of BCAAT, as well as the active form of BCODH, i.e. the activity obtained when the phosphorylation state *in vivo* is preserved. In addition, the activity state of BCODH was measured, which is the percentage of the total activity, or activity after complete dephosphorylation, which is in the active form.

MATERIALS AND METHODS

Sources of materials

Reagents were obtained as previously described [6,7], or from Sigma Chemical Co., St. Louis, MO, U.S.A. Sodium 3-methyl-2-oxo[1-¹⁴C]butanoate, L-[1-¹⁴C]leucine and L-[1-¹⁴C]valine were from Amersham Corp., Arlington Heights, IL, U.S.A. The broad-specificity phosphoprotein phosphatase was prepared as previously described [7,8].

Dated Suffolk sheep of gestational age 120–127 days were studied. Animals were housed in the animal-care facility of the Indiana University Medical Center and cared for according to NIH guidelines. Fed animals were allowed free access to hay and oats; starved animals were provided with water and NaCl for 5 days before study.

Abbreviations used: BCODH, branched-chain 2-oxo acid dehydrogenase; BCAAT, branched-chain amino acid aminotransferase; BCAA, branched-chain amino acid.

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Laparotomy was performed under pentobarbital anaesthesia and the fetus was removed by caesarean section for appropriate tissue sampling. Tissues were removed within 5 min and freeze-clamped with Wollenberger clamps precooled in liquid N₂, or dropped into liquid N₂. Muscle samples were taken from psoas. Adipose-tissue samples were subcutaneous, taken from the flank. Tissues were stored at -70 °C until further analysis.

Methods

Preparation of tissue homogenates and assay of BCODH activity were as previously described [9]. Conditions for complete activation of BCODH by the broad-specificity phosphoprotein phosphatase were established for each tissue studied. Preincubation of placental extracts with the phosphatase resulted in a rapid loss in activity, presumably owing to high proteolytic activity of this tissue; therefore the activity state of BCODH in placenta could not be determined by this method.

BCAAT and BCODH activities were determined in the same tissue extracts. BCAAT was assayed in a final volume of 2 ml containing 50 mM-Tris/HCl, 5 mM-2-oxoglutarate and 80 µM-pyridoxal phosphate, pH 8.6 at 30 °C. Reactions were initiated with 5 mM-[1-¹⁴C]leucine (5 µCi/mmol) or 10 mM-L-[1-¹⁴C]valine (2.5 µCi/mmol), and terminated 15 or 20 min later with addition to the reaction medium of 2 ml of 2 M-H₂SO₄ saturated with ceric sulphate to decarboxylate 2-oxo acids. The ¹⁴CO₂ collected in this manner therefore accounts for all the 2-oxo acid formed by BCAAT (i.e. that released into the medium plus that decarboxylated by BCODH). All other conditions of the assay and CO₂ collection were as in the BCODH assay. Linearity with respect to incubation time and amount of tissue extract used in the assay was established for each tissue studied for both BCODH and BCAAT activities. Statistical analysis was by Student's *t* test for unpaired data.

RESULTS AND DISCUSSION

Rates of transamination of leucine and valine were nearly the same in the tissues studied. Therefore only activities with leucine are given in Table 1. This indicates that isoenzyme II of BCAAT, which preferentially uses leucine [10], is not the major isoenzyme responsible for transamination in any of the tissues studied, because activities with leucine were not larger than activities with valine.

Although the tissue distribution of BCAAT in fetal sheep was similar to that of the rodent (i.e. high in skeletal muscle and low in liver), this was not the case in the mother. The most striking finding was that maternal skeletal muscle was nearly devoid of this activity (Table 1). This indicates a major difference in the inter-organ metabolism of the BCAAs in the adult sheep as compared with the rodent, in which branched-chain 2-oxo acids are derived primarily from peripheral transamination and are subsequently oxidized to a large extent in liver [11]. Further, the low skeletal-muscle BCAAT activity was not an adaptation of pregnancy in the ewe: leucine transaminase activities in non-pregnant ewes were 8 ± 2 and 4 ± 1 nmol/min per g wet wt. in the fed and starved states respectively (means ± S.E.M. for four observations). Such low skeletal-muscle BCAAT activity in adult sheep (at least 100 times lower than that of adult rat [10]) is consistent with previous observations

Table 1. Leucine transaminase activities in maternal and fetal tissues

Activities (nmol/min per g wet wt. of tissue) were determined as described in the Materials and methods section, and are expressed as means ± S.E.M. for four to seven observations. There was no significant difference between the fed and starved states; **P* < 0.01 versus mother.

Tissue	Nutritional state	Maternal activity	Fetal activity
Liver	Fed	11 ± 3	40 ± 5*
	Starved	20 ± 6	40 ± 7
Kidney	Fed	91 ± 2	81 ± 7
	Starved	84 ± 5	84 ± 5
Skeletal muscle	Fed	4 ± 1	200 ± 40*
	Starved	1 ± 1	170 ± 50*
Adipose	Fed	6 ± 1	84 ± 3*
	Starved	1 ± 1	61 ± 5*
Placenta	Fed		2400 ± 300
	Starved		2200 ± 100

of low rates of utilization of the BCAAs by sheep hindquarters *in vivo* [12]. Low BCAAT activity has also been found in muscle from wethers [13], as well as low rates of leucine transamination in muscle preparations from wethers [14]. The difference between sheep and rodents may be a manifestation of the different nutritional adaptation between these two species, i.e. sheep are herbivores and ruminants, whereas rats are omnivores. Perhaps rats would be expected to experience periods of greater BCAA excess in their diet, making it mandatory to have a well-developed mechanism for disposal of these potentially toxic compounds.

It is suggested that lower BCAAT activities in maternal tissues, as compared with the corresponding fetal tissues (particularly low maternal skeletal-muscle activity), may limit maternal oxidation of the BCAAs to the extent that they are made available for fetal and/or placental utilization. Alternatively, higher BCAAT activities in fetal tissues may promote preferential utilization of the BCAAs by the fetus.

The placenta had a high BCAAT activity (Table 1), but was rather poor in BCODH (Table 2). In this respect, placenta was like sheep fetal and rat skeletal muscle, and may play a similar role in BCAA metabolism to that generally ascribed to rat skeletal muscle. The enzyme activities in placenta suggest that net BCAA uptake by the uteroplacental unit observed previously [4] reflects transamination and release of the corresponding 2-oxo acids. Whether these 2-oxo acids are delivered to the mother and/or fetus has not been determined. If they are delivered to the mother, than BCAAT is not likely to limit the extent of maternal BCAA metabolism. On the other hand, preferential uptake of the 2-oxo acids by the fetus would serve to spare the carbon skeleton of this group of essential amino acids for fetal consumption, without enhancing the already high fetal nitrogen load. The branched-chain 2-oxo acids could then be used by the fetus either as an energy source or anabolically through the reversible BCAAT.

Table 2. Branched-chain 2-oxo acid dehydrogenase active form, total activity and activity state in fetal and maternal tissues

Activities (nmol/min per g wet wt. of tissue) were determined as described in the Materials and methods section, and are expressed as means $\pm$ S.E.M. for five to ten observations. The activity state is $100 \times$ activity of the active form divided by the total activity.

* $P < 0.01$ versus the corresponding maternal tissues; † $P < 0.01$ versus fed; ND, not determined.

Tissue	Nutritional state	Activity of active form	Total activity	Activity state (%)
Fetal				
Liver	Fed	130 $\pm$ 20*	290 $\pm$ 30	45 $\pm$ 5*
	Starved	140 $\pm$ 20	300 $\pm$ 30*	48 $\pm$ 6*
Kidney	Fed	70 $\pm$ 5*	78 $\pm$ 12*	100 $\pm$ 10
	Starved	93 $\pm$ 9*	99 $\pm$ 11*	100 $\pm$ 10*
Skeletal muscle	Fed	10 $\pm$ 1	26 $\pm$ 2	38 $\pm$ 5
	Starved	7 $\pm$ 1	25 $\pm$ 3	27 $\pm$ 2
Adipose	Fed	24 $\pm$ 5*	ND	ND
	Starved	15 $\pm$ 8	ND	ND
Placenta	Fed	19 $\pm$ 3	ND	ND
	Starved	15 $\pm$ 1	ND	ND
Maternal				
Liver	Fed	280 $\pm$ 30	330 $\pm$ 20	87 $\pm$ 7
	Starved	96 $\pm$ 14†	93 $\pm$ 14†	98 $\pm$ 6
Kidney	Fed	290 $\pm$ 20	360 $\pm$ 30	78 $\pm$ 5
	Starved	200 $\pm$ 20†	300 $\pm$ 10†	64 $\pm$ 4
Skeletal muscle	Fed	9 $\pm$ 1	18 $\pm$ 2	52 $\pm$ 6
	Starved	8 $\pm$ 1	19 $\pm$ 2	44 $\pm$ 5
Adipose	Fed	5 $\pm$ 1	ND	ND
	Starved	2 $\pm$ 1†	ND	ND

BCODH activity was highest in liver and kidney in both the mother and the fetus (Table 2). In the fed state, BCODH activity was considerably higher in maternal liver and kidney as compared with the corresponding fetal tissues. There was no significant difference in the activity or activity state of BCODH between the fed and starved states in any of the fetal tissues studied.

In contrast with the fetus, BCODH activities in maternal tissues were significantly lower in liver, kidney and adipose after starvation, which occurred in the absence of any significant change in the activity state of the enzyme in liver and kidney (Table 2). The change in BCODH activities in maternal tissues may in part explain the observation that the blood concentrations of the BCAAs typically increase almost 2-fold in both the mother and fetus during maternal starvation [5]. That is, decreased maternal catabolism of the BCAAs associated with decreased maternal BCODH activity may contribute to the increase in BCAAs during starvation, resulting from proteolysis. This would serve to spare the BCAAs or the corresponding 2-oxo acids for fetal consumption. In addition, this would imply that BCODH becomes at least partially rate-limiting for maternal BCAA catabolism during maternal starvation.

Elevated fetal blood concentrations of the BCAAs with maternal starvation may promote flux of these substrates through the fetal BCAAT and BCODH, which remain active during maternal starvation. This may explain the increased arteriovenous difference for leucine across the fetal hindlimb with maternal starvation [15], even though no significant differences in enzyme activities were observed in fetal skeletal muscle. Similarly, the lack of detectable change in enzyme activities in any

of the fetal tissues studied does not preclude enhanced oxidation of the BCAAs by the fetus during maternal starvation. On the contrary, it suggests that the BCAAs may contribute to the general increase in fetal amino acid oxidation with maternal starvation and that the rate of fetal BCAA oxidation may be dictated largely by substrate availability.

In summary, the fetus may extract the BCAAs or their corresponding 2-oxo acids from the maternal circulation, owing to the higher BCAAT activity in fetal tissues. They may be further spared for fetal consumption as a result of decreased maternal BCODH activity during a period of compromised maternal nutrition. It seems likely that net placental uptake of the BCAAs results from transamination and release of the corresponding 2-oxo acids. Finally, fetal oxidation of the BCAAs may be regulated primarily by substrate availability rather than by the activities of fetal BCAAT or BCODH.

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