Tissue-Specific Responses of Branched-Chain α-Ketoacid Dehydrogenase Activity in Metabolic Acidosis

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Abstract. In adrenalectomized rats, acidosis does not increase whole-body leucine oxidation unless a physiologic amount of glucocorticoids (dexamethasone) is also provided; an equivalent dose of dexamethasone without acidosis does not change leucine catabolism. Because the influences of acidification and glucocorticoids on branched-chain amino acid metabolism in specific organs are unknown, the function of branched-chain α-ketoacid dehydrogenase (BCKAD), the rate-limiting enzyme in branched-chain amino acid catabolism, in adrenalectomized rat skeletal muscle and liver, the two major tissues that degrade branched-chain amino acid was measured. In muscle of acido tic adrenalectomized rats receiving dexamethasone, basal and total BCKAD activities were increased 2.6- (P < 0.05) and 2.8-fold (P < 0.05), respectively. Neither acidosis nor dexamethasone alone increased these activities. BCKAD E1α subunit mRNA in muscle of acido tic rats given dexamethasone was increased 1.89-fold (P < 0.05) in parallel with the change in BCKAD activity; BCKAD E2 subunit mRNA was increased by acidosis, dexamethasone, or a combination of both stimuli. In contrast, basal BCKAD activity in liver of rats with acidosis or dexamethasone was nearly threefold lower (P < 0.05) and changes in enzyme activity reflected reduced subunit mRNA. Thus, there are reciprocal, tissue-specific changes in BCKAD function in response to acidosis.

Several clinically relevant conditions are associated with changes in branched-chain amino acid (BCAA) metabolism. In normal adults or experimental animals, restricted dietary protein suppresses amino acid oxidation, leading to improved essential amino acid utilization (1–4). In patients or rats with chronic renal failure, levels of BCAA in the muscle intracellular free amino acid pool and plasma are low, compatible with accelerated metabolism of these essential amino acids (5,6). Bergstrom and colleagues found an inverse correlation between the free valine concentration in muscle and predialysis plasma bicarbonate concentrations in hemodialysis patients without protein malnutrition (6). This group recently reported that when hemodialysis patients were given oral bicarbonate supplements for 6 months, their mean blood bicarbonate increased from 20.6 to 25.9 mM and BCAA and total essential amino acids in muscle were significantly higher (7). These studies are consistent with the conclusion that acidosis stimulates BCAA degradation in muscle. In fact, rates of leucine oxidation are higher in rats and humans with chronic renal failure complicated by acidosis (5,8,9). Moreover, induction of metabolic acidosis in normal adults (mean plasma bicarbonate, 18 ± 1 mM) or rats by NH4Cl ingestion (mean plasma bicarbonate, 12 ± 1 mM) has been shown to increase whole-body amino acid oxidation (10,11).

During an investigation of the mechanism of increased BCAA oxidation in muscle, we found that acidosis (mean plasma bicarbonate, 9 ± 1 mM) increases the activity of the rate-limiting enzyme, branched-chain α-ketoacid dehydrogenase (BCKAD) (12,13), which is a multimeric complex composed of E1α, E1β, E2, and E3 subunits (14). These reports, however, do not identify what signals activate BCAA degradation and whether this occurs uniformly in all organs.

What factors could increase BCAA catabolism? May and colleagues studied adrenalectomized rats and found that both acidosis and glucocorticoids must be present before BCAA oxidation increases in vivo (15). Because amino acid oxidation was measured in vivo, the results reflect the weighted average of BCAA metabolism in the major organs that metabolize BCAA, including liver and muscle. It has also been reported that glucocorticoids can stimulate BCKAD activity in cultured liver cells (16). Consequently, it seemed reasonable that the same responses occur in liver as in muscle. We have investigated the effects of acidosis and glucocorticoids on BCKAD function in muscle and liver from adrenalectomized rats.

Materials and Methods
Rat Model
All studies were conducted in accord with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Sprague-Dawley rats, each weighing approximately 75 g, underwent bilateral adrenalectomy (ADX) and recovered from surgery; for the next 2 weeks, rats were allowed to eat a 23% protein chow diet and to drink 0.9 g/dl saline. Dexamethasone treatment and induction of metabolic acidosis were performed as described (15,17) with minor modifications. Briefly, acidic rats were fed a 14% protein diet containing NH4Cl twice daily by gavage and were given a 0.25 M NH4Cl/0.45 g/dl saline solution to drink ad libitum; nonacidotic rats were given an identical diet and fluid without NH4Cl. To study the influence of glucocorticoids, control (n = 8) and acido tic (n = 12) rats were given 10 μg dexamethasone/100 g body wt per d by subcuta-
Acidosis and Branched-Chain Amino Acids

To study the influence of acidosis without glucocorticoids, ADX rats (n = 12) were given NH₄Cl only. Nine days after initiating the protocols, arterial blood was taken from anesthetized rats (fasted overnight) to measure serum bicarbonate concentrations. These values more accurately reflect acid-base status than arterial blood pH because there can be acute changes in PCO₂ with anesthesia (18). Gastrocnemius muscles and liver were also removed and frozen in liquid nitrogen.

**BCKAD Activity Measurement**

BCKAD enzyme activity was determined by measuring the rate of decarboxylation of [1-¹⁴C] α-ketoisocaproate (KIC). In muscle, basal and total BCKAD activities were measured with a partially purified enzyme preparation as described (13). Basal BCKAD in liver was measured by pulverizing/homogenizing frozen tissue in 1.2 ml of Hepes buffer containing diithiothreitol, inhibitors of proteases, kinases, and phosphatasess. The homogenate was then incubated in “assay buffer” containing thiamine, coenzyme A, dithiothreitol, NAD, and MgCl₂, and 4.5 mM KIC (specific activity, 200 μCi [1-¹⁴C] KIC/ mmol KIC). ¹⁴CO₂ was collected and measured (13).

**Measurement of BCKAD Subunit mRNA**

Total RNA from muscle or liver was isolated using TriReagent (Molecular Research Center, Cincinnati, OH). RNA was separated by agarose gel electrophoresis under denaturing conditions and transferred to a ZetaProbe nylon hybridization membrane (BioRad Laboratories, Hercules, CA). The blots were hybridized with cDNA probes of the human BCKAD E₁α subunit or rat glyceraldeyde-3-phosphate dehydrogenase (GAPDH) or an antisense RNA probe of the rat BCKAD E₂ subunit as described (13,17). Autoradiographic bands were quantified using a BioRad model 05-670 densitometer. To correct for minor differences in loading, each BCKAD subunit value was normalized by dividing it by the corresponding value of GAPDH mRNA. We have shown that adrenalectomy with or without acidosis, glucocorticoids, or both, does not affect GAPDH mRNA levels in muscle (17).

**Measurement of Mitochondrial BCKAD Proteins**

BCKAD E₁α, E₁β, and E₂ subunit proteins in isolated mitochondrial protein fractions from liver were measured using Western blotting techniques and a polyclonal antibody to the BCKAD complex as described (13,19,20). Levels of BCKAD subunit protein contents in muscle were not measured because in earlier studies, there were no detectable increases in the levels of these proteins in muscles of acidic rats (13).

**Statistical Analyses**

Results are reported as mean ± SEM. An analysis of variance was used to test for statistical significance (P < 0.05) between experimental groups. When significance was found, pair-wise comparisons were performed using Tukey’s t test.

**Results**

Rat weights at the time of tissue harvest were 236 ± 6 g for untreated ADX (n = 6), 238 ± 11 g for acidic ADX (n = 6; P = NS), 195 ± 4 g for dexamethasone-treated ADX (n = 7; P < 0.05 versus untreated ADX), and 200 ± 5 g for acidic, dexamethasone-treated ADX rats (n = 6; P < 0.05 versus untreated ADX). Consistent with previous results (15,18), the serum HCO₃⁻ of untreated ADX rats and ADX rats given dexamethasone were not different (HCO₃⁻ = 24.4 ± 0.4 and 23.1 ± 0.7 mM, respectively; P = NS), whereas the values of NH₄Cl-fed and NH₄Cl-fed, dexamethasone-treated ADX rats were equally acidic (HCO₃⁻ = 12.5 ± 1.4 and 12.8 ± 1.7 mM, respectively; P < 0.01 versus control).

**BCKAD in Muscle**

In muscles of ADX rats, basal BCKAD activity was unchanged by glucocorticoids or by acidification but was higher (P < 0.05) in muscles of acidic ADX rats that had received glucocorticoids (Table 1). When total BCKAD activity (i.e., after dephosphorylation by adding Mg⁺²) was measured in the same rat muscle enzyme preparations, there was no increase in activity in ADX rats given dexamethasone (Table 1). Total BCKAD activity in acidic ADX rat muscle was nearly double that measured in the untreated ADX rat preparation but this increase was not statistically significant. When acidic ADX rats were given dexamethasone, total enzyme activity in muscle was nearly threefold higher (P < 0.05) than in control or glucocorticoid-treated ADX rats.

**Table 1.** BCKAD activity in ADX rat muscle and liver

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver Basal (nmol/mg per min)</th>
<th>Muscle Basal (nmol/mg per min)</th>
<th>Total (nmol/mg per min)</th>
<th>% Activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.65 ± 0.22</td>
<td>15.9 ± 7.8</td>
<td>459 ± 144</td>
<td>3.2 ± 0.9</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>0.23 ± 0.03ᵇ</td>
<td>8.7 ± 1.8</td>
<td>348 ± 124</td>
<td>3.3 ± 0.7</td>
</tr>
<tr>
<td>Acidosis</td>
<td>0.16 ± 0.02ᵇ</td>
<td>15.1 ± 4.1</td>
<td>841 ± 127</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>Acidosis + dexamethasone</td>
<td>0.19 ± 0.06ᵇ</td>
<td>41.3 ± 7.8ᵇ</td>
<td>1305 ± 253ᵈ</td>
<td>3.8 ± 0.6</td>
</tr>
</tbody>
</table>

ᵃ Basal and total BCKAD activity were measured in muscle from ADX rats that were acidic or nonacidic; some rats received 10 μg of dexamethasone/100 g body wt per d. Only basal BCKAD activity was measured in liver of ADX rats (see Results section). Results are reported as means ± SEM (n ≥ 6 rats/group). BCKAD, branched-chain α-ketoacid dehydrogenase; ADX, adrenalectomized.

ᵇ P < 0.05 versus untreated ADX.

c P < 0.05 versus all other treatments.

d P < 0.05 versus untreated or dexamethasone-treated ADX rats.
The steady-state level of mRNA encoding the E2 BCKAD subunit in muscle was increased 109% ($P < 0.01$ versus ADX control) with dexamethasone and 87% ($P < 0.01$ versus ADX control) by acidosis (Figure 1). When acidotic ADX rats were given glucocorticoids, E2 mRNA in muscle was 162% higher than in control ($P < 0.01$ versus untreated ADX control, or acidotic ADX rats). As in our earlier report, GAPDH mRNA in muscle was unchanged by these treatments (data not shown) (17).

We were surprised to detect two E1α mRNA (1.6 and 1.8 kb) in muscle (Figure 1). The larger E1α mRNA (1.8 kb) was not significantly increased by either acidification or dexamethasone alone, but when these two stimuli were combined, there was a significant increase in this mRNA species over the level measured in muscles of control (88%; $P < 0.01$), dexamethasone-treated (37%; $P < 0.01$), or acidotic (56%; $P < 0.05$) ADX rat muscles. The smaller mRNA species (1.6 kb) was unchanged by dexamethasone, acidosis, or the combination of treatments.

**BCKAD in Liver**

The basal BCKAD activity was highest in livers of untreated ADX rats but was diminished by dexamethasone treatment (Table 1). Acidification alone also decreased enzyme activity in liver; the combination of acidosis plus dexamethasone did not reduce BCKAD activity further. We attempted to determine whether glucocorticoids or acidosis influence total BCKAD activity in ADX rat liver by incubating the BCKAD enzyme preparation with a bovine heart phosphatase preparation as described by Harris and colleagues and Chicco et al. (16, 21, 22). The heart phosphatase preparation has been used as a means to estimate the total BCKAD activity through dephosphorylation of the E1α subunit of BCKAD (21, 22). Our phosphatase preparations from bovine heart repeatedly failed to increase BCKAD activity isolated from ADX rat liver. Moreover, commercially available phosphatases did not increase BCKAD activity above the basal level. Regardless, BCKAD activity is almost completely activated (dephosphorylated) in liver of normal rats whereas in muscle, only 3 to 5% of BCKAD activity is normally in the active state (21, 23). This finding suggests that basal BCKAD activity in liver closely approximates total enzyme activity.

To determine whether there is a coordinated response between BCKAD activity and subunit mRNA levels in liver that is similar to the relationship found in muscle of ADX rats and intact rats (13), we measured the levels of BCKAD subunit mRNA in liver. In contrast to muscle, a single E1α mRNA was detected in liver (Figure 2) with a size (1.8 kb) similar to that reported by others (13, 24). Acidosis tended to reduce hepatic E1α and E2 mRNA levels (17% and 25%, respectively; $P = \text{NS}$), whereas dexamethasone reduced them by 39% ($P < 0.05$ versus untreated ADX rats) and 22% ($P = \text{NS}$), respectively (Figure 2). When acidification and dexamethasone were combined, the levels of E1α and E2 mRNA were diminished 49% ($P < 0.05$ versus control or acidotic ADX rats) and 35% ($P < 0.05$ versus untreated ADX controls), respectively. The levels of hepatic GAPDH mRNA were not altered significantly by acidosis (0.13 ± 0.01 densitometric units in untreated ADX rat liver compared with 0.15 ± 0.01 in acidotic ADX rat; $P = \text{NS}$) dexamethasone (0.12 ± 0.01), or the combination of the two treatments (0.13 ± 0.01).

The levels of hepatic BCKAD proteins in liver mitochondria were compared using Western blotting techniques (Figure 3). In acidotic ADX rats, there was no change in the levels of E1α,
E1α, and E2 subunit proteins. In contrast, dexamethasone treatment in the absence of acidosis reduced the levels of these subunits 25%, 20%, and 20%, respectively (P < 0.05 for each versus untreated ADX controls). With acidosis plus glucocorticoids, hepatic BCKAD subunit proteins were reduced further (E1α, -37%; E1β, -24%; and E2, -29%; P < 0.05 versus untreated ADX controls). These latter values were not statistically different from those in ADX rats receiving dexamethasone alone.

**Discussion**

In patients and rats, acidosis is a stimulus causing BCAA catabolism, however, acidosis also raises plasma cortisol, and pharmacologic doses of dexamethasone also stimulate BCKAD activity in rat muscle (5,8,25,26). This finding raises a question about which stimulus (i.e., glucocorticoids or acidosis) increases BCAA degradation. May and colleagues investigated this question by measuring leucine oxidation in intact ADX rats (15). They found that leucine oxidation in vivo was twofold higher in acidic ADX rats given dexamethasone compared with the rate measured in untreated ADX rats. Leucine oxidation in acidic ADX rats or nonacidotic ADX rats given dexamethasone was not increased. They concluded that both acidosis and glucocorticoids were required to stimulate BCAA oxidation, but specific organ responses to these stimuli were not identified. To address this issue, we have measured BCKAD activity in the two major organs that degrade branched-chain amino acids, liver and muscle (14). Reciprocal, tissue-specific changes in both BCKAD activity and subunit mRNA levels were found in the presence of these two stimulii.

The responses to acidosis, glucocorticoids, or both, found in muscle are analogous to the “whole-body” responses measured by May *et al.* (15). It seems that the increase in basal activity in muscles of acidic ADX rats given dexamethasone primarily is attributable to an increase in total BCKAD activity, because the percentage activation was unchanged. Moreover, the level of E1α mRNA in muscle was increased only when there was a combination of acidosis plus glucocorticoids, reflecting changes in the measured enzyme activity. Why the level of E2 mRNA increased significantly with acidosis or glucocorticoids alone is not apparent. It is important to note that the BCKAD complex is proposed to consist of four pairs of E1α/E1β subunit pairs surrounded by 24 E2 subunits (27). BCKAD subunit proteins are encoded in the nucleus, translated in the cytoplasm and imported into the mitochondria for assembly of the active enzyme complex. The rate-limiting step for the formation of functional BCKAD complexes is unknown. If acidosis or dexamethasone were to change the rates of translation, import, or assembly, there could be discrepancies between subunit mRNA levels and BCKAD activity. Our most important finding, however, is that the greatest increase in E1α and E2 mRNA were found in acidotic ADX rats that also received dexamethasone. Because these rats have the highest BCKAD activity in muscle, they are most representative of the in vivo state. It is tempting to speculate that the E1α subunit may be important in determining the activity of BCKAD in muscle because basal and total activities are increased only when there is an increase in the level of E1α subunit mRNA (reference 13 and unpublished data, S.R. Price).

It is unclear why two species of E1α mRNA (1.6 and 1.8 kb) were detected in muscle (Figure 1). In earlier work, only a single 1.8-kb E1α mRNA was detected with a short antisense E1α RNA in muscle (13). The different results might be
In liver, glucocorticoids or acidosis act to suppress BCKAD activity, but the combination of acidosis and dexamethasone did not reduce this activity further. The lower BCKAD activity in liver of dexamethasone-treated ADX rats was unexpected based on the report of Chicco et al., who found that dexamethasone increases BCKAD activity, subunit mRNA and subunit proteins in primary cultures of hepatocytes (16). However, their results were obtained by adding an analogue of cAMP plus dexamethasone to the culture media. Thus, interpretation of how glucocorticoids influence hepatic BCKAD activity is complicated. Importantly, others have found that results obtained with isolated hepatocytes can differ from those in intact liver tissue. For example, interleukin-1 induces the synthesis of hepatic "nonsecretory" proteins in isolated hepatocytes but not in perfused rat livers (30). These types of experimental discrepancies underscore the importance of confirming results obtained with cultured cells in experimental animal models.

The influence of metabolic acidosis on BCAA exchange or oxidation in liver is presently unknown. Alvestrand and colleagues found that splanchnic uptake of BCAA is increased in chronic renal failure patients, but they were not acidotic (mean serum bicarbonate = 23 mM) (31). In liver of rats with 
\[ \text{NH}_4\text{Cl-induced chronic acidosis, overall amino acid uptake is increased compared with control rat liver, but BCAA uptake was not measured (32). Because acidosis accelerates BCAA catabolism in muscle, the contribution of BCAA released from muscle to the intracellular free BCAA pool in liver would be reduced. Therefore, suppression of hepatic BCKAD activity would help preserve intracellular free BCAA concentrations in liver for anabolic processes like the synthesis of acute phase proteins.}

The changes in BCAA catabolism that occur in muscle and liver of lipopolysaccharide (LPS)-treated rats are similar to those measured in ADX-acidotic rats receiving dexamethasone (33–35). BCKAD activity in muscle is increased by LPS treatment (33,34), but hepatic BCAA uptake and oxidation are diminished (35). Moreover, plasma BCAA levels are diminished and whole-body leucine oxidation is increased in rats treated with LPS (33). Thus, BCAA seems to be predominantly catabolized in muscle, but in liver, BCAA are used for anabolic processes in catabolic conditions.

In summary, acidosis causes reciprocal changes in the activity of BCKAD, the rate-limiting enzyme in the oxidative degradation of BCAA, in liver and muscle. These opposing responses to the same external stimuli seem to change the utilization of BCAA in different organs. If similar responses occur in humans, they could explain why acidotic hemodialysis or CAPD patients do not exhibit increased whole-body BCAA oxidation (36,37). Finally, our results support the contention that muscle is the major organ where BCAA are metabolized in acidosis, because plasma BCAA pools are not fully corrected, despite depressed BCKAD activity in liver.

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References


