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Modulation of Branched-Chain Amino Acid Metabolism by Exercise in Rats

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ABSTRACT

A variety of important roles for branched-chain amino acids in metabolic regulation has been suggested. Branched-chain α-keto acid dehydrogenase(BCKAD) complex is a rate limiting enzyme in branched-chain amino acid metabolism. The purpose of this study was to examine the effects of exercise on the activity and activity state of branched-chain α-keto acid dehydrogenase in rat heart and liver tissues. Forty-eight Sprague-Dawley rats were assigned into three experimental groups : sedentary control, exercised, or exercised-rested. Submaximal exercise(running) for two hours significantly increased basal activity without a change in total activity in both tissues, with a concomitant increase in activity state of the enzyme complex. At 10 min post-exercise, heart enzyme activity significantly decreased, though not to the control level, while liver enzyme activity remained unchanged. These data suggested that the exercise-induced increase in branched-chain α-keto acid decarboxylation in rat tissues may not be the result of enzyme synthesis, but rather is due to increased activity of the BCKAD.

KEY WORDS : branched-chain amino acid · exercise · branched-chain α-keto acid dehydrogenase activity · rat heart · rat liver.

Introduction

Numerous studies on both humans and animals strongly support that branched-chain amino and keto acids play a potential role in the regulation of carbohydrate, lipid, and protein metabolism[1-3]. Branched-chain α-keto acid dehydrogenase(BCKAD) is a rate limiting enzyme which catalyzes the oxidative decarboxylation of the branched-chain keto acids. Branched-chain α-keto and amino acids are an important source of energy metabolites to cells in the absence of adequate glucose substrate under several types of physiological conditions such as exercise, fasting, and trauma[4-6].

It has been suggested that exercise induced the alteration of protein metabolism in ways such as decreased nitrogen balance[7][8], increased urea production[9-11], and increased leucine oxidation rate[12][13]. These findings suggest increased amino acid oxidation during exercise, despite the opinion of other investigators that catabolism of protein makes no contribution to energy expenditure [14].

The change of activity state of heart muscle

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BCKAD in response to exercise remains unclear, while the enzyme activity of skeletal muscle has been studied by many investigators[15-18]. Recently, Hutson et al.[19] determined the activity of branched-chain amino transferase(BAT) in mitochondria isolated from rat heart and suggested that BAT activity is located exclusively in the mitochondrial compartment in rat heart. This data suggested that considerable amounts of leucine may be metabolized by the heart. The amount of BCKAD complex of heart and liver may not be large in terms of total body activity, however the relative and specific activity of the enzyme is much higher than that of skeletal muscle. The hypothesis of this study was that BCKAD complex of heart may be activated and thus oxidize more branched-chain keto acids as heart muscle contracts more rapidly to provide increased blood circulation during exercise. The hypothesis was tested by designing the experiment to investigate the effect of exercise on the activity and activity state of BCKAD in rat heart and liver.

Methodology

1. Experimental Animals

Forty-eight male Sprague-Dawley rats weighing 170−200g were randomly assigned into three experimental groups ; sedentary control, exercised, and exercised-rested. Rat chow diet was fed ad libitum throughout the experimental period. The temperature(20−23℃) and humidity were controlled, and lights were automatically timed to be on from 06.00−18.00h.

2. Exercise Training Protocol

After a three-days adaptation period, the animals assigned to exercised and exercised-rested groups were trained(5X/wk) to run on a motor-driven treadmill with an increasing intensity and duration of exercise so that they could accomplish a exercise regimen, 0% grade at 28m/min for 2 hours at the end of the training period. The initial speed was 50%(14m/min) of the final goal, and the initial length of training session was 30 min. The increments of speed and duration of a treadmill running were determined depending on the ability of animals to run. During the entire training period, the rats in control sedentary groups were also brought to the treadmill room and remained in their cages without food while others were running. After two weeks of exercise training, rats in exercised and exercised-rested groups ran on a treadmill with 0% grade at 28m/min for 2 hrs. The rats in the exercised group were sacrificed immediately after exercise, whereas those in the exercised−rested group were rested for 10 min before they were sacrificed to determine the time course of BCKAD activity during recovery from exercise.

3. Tissue Preparation and Enzyme Assay

The BCKAD activity is regulated by the covalent modification via phosphorylation(inactive form) and dephosphorylation(active form)[20-23]. Therefore, it is crucial that the least amount of time should be spent to remove the tissues and that all assays must be done at cold temperature to minimize the interconversion of the enzyme. Only fresh tissues were used in this experiment. Frozen tissues have been rountinely used for the determination of BCKAD activity levels in other studies. In a preliminary experiment of this study, however, freezing the tissue had a significant negative effect on the activity of the enzyme(data not shown). After the rats were euthanized by decapitation using a guillotine, liver and heart were surgically removed and kept ice-cold. The homogenizing buffer was prepared as in Table 1. About 0.3g of fresh liver and heart tissues was

Table 1. Components of homogenizing and assay buffer

Homogenizing buffer (pH 7.8)		Assay buffer (pH 7.4)	
Ingredient	mM	Ingredient	mM
Sucrose[1]	200	Mannitol[2]	200
KCl[1]	50	Sucrose[1]	70
$MgCl_2$[1]	5	$MgCl_2$[1]	5
α-chloroisocaproate[3]	0.1	Cocarboxylase[2]	0.45
Ethyleneglycol-bis(β-aminoethyl-ether)-N,N,N'-tetraacetic acid[2]	5	Coenzyme A[2]	0.8
Tris-(hydroxymethyl)aminomethane[2]	50	NaF[2]	50
NaF[2]	50	N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid[2]	2
N-α-tosyl-l-lysine chloromethylketone[2]	1	NAD^+[2]	2
DL-dithiothreitol[2]	5	DL-dithiothreitol[2]	5
Triton X-100[4]	1mg/ml	Triton X-100[4]	1mg/ml
Trypsin inhibitor[2]	1mg/ml		

1) Mallinckrodt, INC., Paris, KY
2) Sigma Chemical Co., St. Louis, MO
3) Gift from Drs. Strohscheim and Simpson of Sandoz Inc., East Hanover, NJ
4) U.S.Biochemical Corp., Cleveland, OH

quickly removed(<30sec) from rat and homogenized with homogenizing buffer on ice. A 10% crude homogenate was routinely prepared for this study, and the activity of BCKAD was determined by measuring the release of $^{14}CO_2$ from α-keto[1-^{14}C]-isovalerate(Amersham) using a modification of the method of Kasperek et al.[21]. Assay buffer(Table 1) was prepared, and the assay itself was performed by incubating a portion of the crude homogenate(100ul liver and heart) made from fresh tissue in 2.0ml assay buffer at 37℃, pH7.4. Homogenizing buffer was added to bring the volume to 2.8ml. The reaction was initiated by adding 50ul α-keto[1-^{14}C]-isovalerate(4mM, 0.01 uCi/50ul). After incubating for 20min the reaction was stopped by adding 0.2ml 25% trichloroacetic acid. The liberated $^{14}CO_2$ was collected by methylbenzothenium hydroxide in a center well for 1hr, and the radioactivity was counted in 7.5ml Scinti Verse E containing 10% water. Blanks were assayed in the same manner except the homogenate was poisoned with 0.2ml 25% trichloroacetic acid prior to addition of the keto acid. Total activity of BCKAD was determined after complete activation of the enzyme in homogenates made from fresh tissue. The homogenizing and assay buffers for total activity were same as the ones used for basal activity except that NaF and Triton X-100 were ommited.

4. Statistical Analysis

All the statistical analyses were performed using the Statistical Analysis System(SAS). Tukey's studentized range test for variable was used to test the differences between means, and the differences were considered significant at $p<0.05$.

Results

1. Enzyme Assay at Different Incubation Time

The BCKAD complex is readily interconvertible between active(dephosphorylated) and inactive(phosphorylated) forms. Therefore, the biggest challenge in the assay of BCKAD activity

is to arrest the interconversion between two forms. To determine if interconversion occurred during the assay, the enzyme activity was measured at different lengths of times on liver and heart homogenates in which the BCKAD had been partially activated by a 10 min preincubation. The linearity of the release of $^{14}CO_2$ with time showed that the activity of the enzyme complex was unchanged during the assay(Fig. 1). If the activity of BCKAD were increasing or decreasing during the assay, the plot would have curved up or down respectively.

2. Total Feed Intake, Body Weight Gain, and Tissue Weight

There were no statistically significant differences of estimated total feed intake per animal among control sedentary, exercised, and exercised-rested rats(Table 2). The total body weight gain during the two week training period, however, was significantly affected by exercise($p=0.0001$). Rats in exercised and exercised-rested groups gained significantly less body weight than those in the control sedentary group. Both liver and heart weight per 100g body weight were significantly influenced by exercise(Table 2).

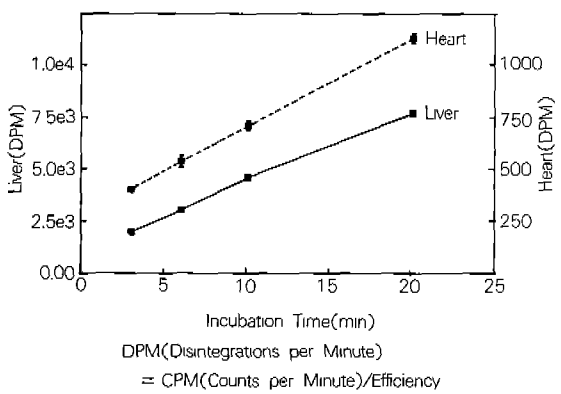

Fig. 1. $^{14}CO_2$ release at different incubation times.

3. BCKAD Activity in Heart and Liver

Basal activity is defined as the enzyme activity determined when the in vivo phosphorylation is preserved. The enzyme activity obtained after complete dephosphorylation, or activation, is defined as total activity.

1) Heart Basal and Total Activity

Basal enzyme activity in heart was significantly increased by 2-hr treadmill running and significantly decreased from the exercise state by a 10min rest after exercise(Fig. 2). The effect of running on the treadmill at 0% grade, 28m/min for 2 hrs was to significantly increase the basal activity of BCKAD in rat heart from 10.9± 4.9 to 63.0± 4.9 nmol/min/g tissue. After 10min recovery from the exercise, the enzyme activity was significantly lowered to 33.3± 5.0nmol/min/g tissue, but not to the level of the control sedentary group (10.9± 4.9 nmol/min/g tissue). However, total activity levels of heart tissue among control sedentary, exercised, and exercised−rested groups were not significantly different, with values of 317.3± 13.7, 285.5± 13.

Table 2. Estimated total feed intake per animal and total body weight gain during a two-week training period and tissue weight of experimental rats

Activity state	Estimated feed intake, g	Body weight gain, g	Tissue weight gm/100g B.W.	
			Heart	Liver
Control	304.0 ± 8.9	112.9 ± 3.3	0.331 ± 0.006	3.40 ± 0.05
Exercised	284.4 ± 8.9	89.5 ± 3.3[a]	0.351 ± 0.006[a]	3.11 ± 0.05[a]
Ex'ed-rested	281.5 ± 8.9	88.5 ± 3.3[a]	0.349 ± 0.006	3.06 ± 0.05[a]

Values are LS Mean± SE for 16 rats in each group

a) Significantly different from sedentary control rats at $p<0.05$

9, and 329.0± 13.7nmol/min/g tissue respectively.

2) Liver Basal and Total Activity

Treadmill running exercise significantly increased basal activity of the liver enzyme, and the enzyme activity at 10min post exercise was not significantly different from that of rats sacrificed immediately after exercise. However, two-hour treadmill running did not significantly influence total enzyme activity in liver(Fig. 3).

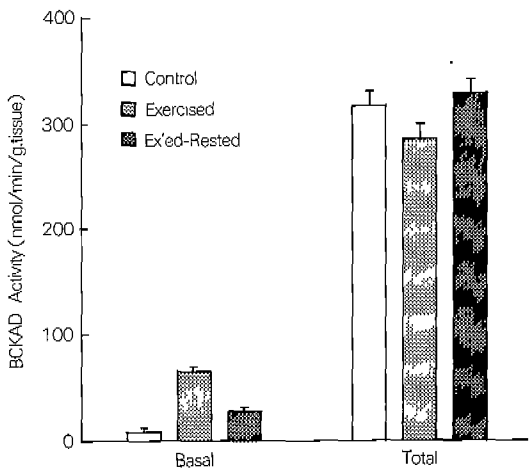

Fig. 2. BCKAD activity in rat heart.

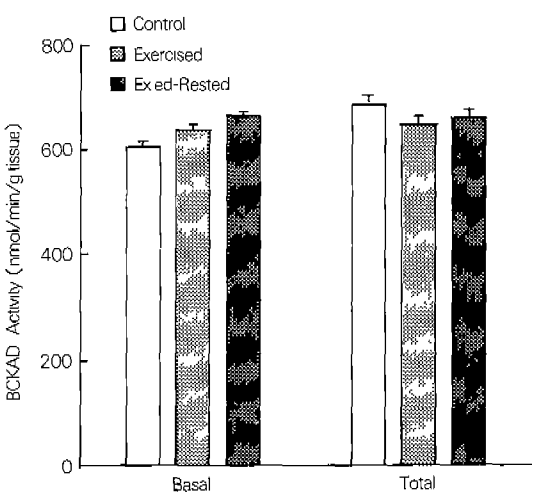

Fig. 3. BCKAD activity in rat liver.

4. BCKAD Activity State in Heart and Liver

Proportion of the BCKAD enzyme present in the active form in the tissue is defined as the activity state of the enzyme, and extrapolated by determining the percentage of basal activity to total activity in the tissues. The activity state of the BCKAD was significantly increased by exercise in both heart and liver(Fig. 4). In sedentary control rats, only 3.3± 2.6% of the heart enzyme was in active form, and the two-hour exercise increased the proportion of the enzyme in active form to 23.6± 2.6%. In 10min post exercise rats, however, the activity state of heart enzyme decreased to 9.9± 2.6%, which was not significantly different from that of the control group. In liver, 83.9± 5.2% of the enzyme was present in the active form in control sedentary rats. Two-hour treadmill running converted virtually all of the enzyme to the active form(99.0± 5.2%), and this change persisted after 10min post-exercise(98.8± 5.2%). The activity states of both exercised and exercised-rested groups were significantly different from that of control rats.

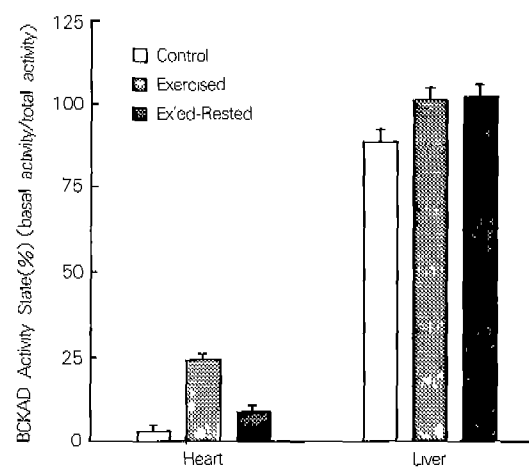

Fig. 4. BCKAD activity state in rat tissues.

Discussion

1. Total Feed Intake, Body Weight Gain, and Tissue Weight

Interesting observations were made on total feed intake and body weight gain. Though the training period was relatively short, the differences of weight gain between exercised and sedentary control rats were visible, meanwhile there were no statistically significant differences of total feed intake among control sedentary, exercised, and exercised-rested rats. Although no data are available to compare, this study showed aerobic submaximal exercise significantly supressed body weight gain compared to the sedentary control animals. These differences in body weight gain due to exercise do not provide deductive evidence whether the trend is transient or valid for a long term training.

Since organ weights may vary depending on the whole body weight, weights were expressed per 100g body weight. There was a tendency for animals in exercised groups to have lower liver weight and higher heart weight than those in control sedentary groups.

2. BCKAD Activity in Heart and Liver

It has been suggested that the rate of leucine oxidation is increased during exercise[16)24-26)]. Though leucine has been the most frequently used substrate for the study of branched-chain amino and keto acids metabolism, the α-keto acid of valine(α-keto isovaleric acid) was used as a substrate for the BCKAD complex in this study because of enhanced sensitivity.

Treadmill running at 0% grade, 28m/min for 2hrs significantly increased the basal activity of BCKAD in rat heart from 10.9± 4.9 to 63.0± 4.9 nmol/min/g tissue, a six-fold increase in α-keto isovalerate decarboxylation. Wagenmakers et al.[26)] has reported modest increases(1.5-fold) of enzyme activity from 23.4nmol/min/g tissue to 34.6 nmol/min/g tissue in heart by 60min treadmill running at 12m/min up a 15° incline. On the other hand, Kasperek et al.[16)] reported much higher levels of basal enzyme activity in heart, 44.6± 5.8 nmol/min/g tissue in resting rats and 217± 25 nmol/min/g tissue in exercising rats, resulting in a 5-fold increase in the activity state of heart enzyme. Possible explanations of the discrepancy in reported values of enzyme activity could be : 1) use of different substrate for enzyme complex such as α-keto isocaproate, 2) freezing of tissue which may affect the enzyme activity, 3) variable intensity and duration of exercise, 4) whether or not rats were trained to perform a assigned exercise regimen, 5) variable contents of assay and homogenizing buffers which may influence the control of enzyme activity, such as kinase, phosphatase, and protease inhibitors. This study supports that the relative importance of heart tissue for the degradation of branched-chain α-keto acids in response to exercise should be recognized. Increases in oxidation of branched-chain α-keto acids in heart by exercise could contribute to provide substrate precursors for the gluconeogenic pathway, thereby may playing an indirect role in the energy-yielding processes in response to increased energy needs during exercise.

To further study the time course of changes in enzyme activity by exercise, rats in the exercised-rested group were allowed to rest for 10min after exercise and assayed for BCKAD activity. While the untrained rats in the study of Kasperek et al.[16)] returned to pre-exercise level of BCKAD activity in skeletal muscle after a 10min rest, trained rats in this study showed only a 47% decrease in maximal exercise-induced enzyme activity at 10min after exercise. This apparent discrepancy

may be explained by : 1) different tissues having different time courses of BCKAD activity. Heart tissue may sustain high enzyme activity even after exercise cessation because of gradual slowing of the muscle contractions, whereas skeletal muscle enzyme activity may return more rapidly to its control resting value as muscle goes into the resting state. 2) Whether or not the rats were trained could make a difference in the ability of tissues to keep the enzyme in the active form. No data are available to compare the time course of BCKAD activity after exercise in heart.

It has been reported that liver BCKAD activity remains virtually unchanged by exercise[16)27)], since nearly full enzyme activity already exists in rat liver prior to exercise. Two-hour treadmill running significantly increased basal activity of liver BCKAD complex. Liver BCKAD activity at 10 min post exercise was not significantly different from that of rats sacrificed immediately after exercise, suggesting that liver tissue may sustain high enzyme activity during the recovery from exercise.

To determine the maximal ability of heart and liver to oxidize the branched-chain keto-acids, the total enzyme activity of BCKAD was measured after incubating the tissue homogenates with NaF and Triton X-100 for complete activation of the enzyme. The total activity of BCKAD was unchanged by exercise in both tissues. These findings support that exercise does not affect enzyme synthesis or degradation, but that the enzyme activity is enhanced by exercise, which agree with other investigators' findings[16)26)].

3. BCKAD Activity State in Heart and Liver

Besides the basal(actual) and total activities per se, activity states of the BCKAD complex which represents the proportion of the enzyme present in the active form in the tissue were determined in this study to explain more clearly how the BCKAD complex responds to exercise. The activity state of BCKAD complex was significantly increased by exercise in both heart and liver. In sedentary control rats, only 2.9± 1.9% of the heart enzyme was in active form, and two-hour exercise increased the proportion of the enzyme in active form to 24.3± 1.9%., resulting in a 8-fold increases in heart enzyme complex in active form by exercise. Wagenmakers et al.[26)] reported a 1.4-fold increase(from 7.8% to 10.8%) of heart enzyme complex in active form by exercise ; Kasperek et al.[16)] suggested a 5-fold increase(from 20% to 100%) of heart enzyme activity state. In 10min post exercise rats, however, the activity state of heart enzyme had decreased to 8.8± 1.9%, a value not significantly different from that of the control group. In liver, 88.5± 3.7% of the enzyme was present in the active form in sedentary rats, which was somewhat lower than the reports of other investigators[16)26-27)], and the effect of exercise was to convert virtually all the enzyme to its active form(101.6± 3.7%). This state persisted through the 10min post exercise period(102.6± 3.7%) suggesting that the liver enzyme activity was more resistant to change by exercise and resting than heart enzyme. Gillim et al.[27)] reported 94% of the liver BCKAD was in active form in rats ; while a 97% active form of liver enzyme was suggested by Wagenmakers et al.[26)] in control rats.

In conclusion, submaximal exercise(running) for two hours significantly increased basal activity without a change in total activity in rat heart and liver tissues, with a concomitant increase in activity state of the enzyme complex. These data suggest that the exercise-induced increase in branched-chain α-keto acid decarboxylation may not be the result of enzyme synthesis, but rather is due to increased activity of the BCKAD. At 10min post-exercise, heart enzyme activity significantly decreased, though not to the control level, while

liver enzyme activity remained unchanged.

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= 국문초록 =

운동에 의한 Branched-Chain 아미노산 대사의 조절에 관한 연구

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사람과 동물을 이용한 여러 연구들에서 branched-chain 아미노산과 케토산이 탄수화물, 지질 및 단백질 대사에 있어서 중대한 역할을 하고 있다는 사실이 밝혀졌다. Branched-chain α-keto acid dehydrogenase는 branched-chain 아미노산의 산화분해를 촉매하는 조절효소이다. 본 연구의 목적은 운동에 의한 쥐의 간 조직과 심장 조직내의 branched-chain α-keto acid dehydrogenase 활성도에 미치는 영향을 조사하기 위함이었다. 48마리의 Sprague-Dawley 쥐를 운동상태에 따라 비운동구(sedentary control), 운동구(exercised) 및 운동후-휴식구(exercised-rested)의 세 그룹으로 나누었으며 운동구와 휴식구에 배치된 쥐들은 본 실험에 들어가기 전 2주동안 exercise training(1주일에 5번)을 받았다. 본 실험에 사용된 운동은 motor가 달린 treadmill(0% grade)에서 분속 28m의 속도로 두시간 동안의 running이었다. 운동구의 쥐들은 운동이 끝난 즉시 희생된 반면 운동후-휴식구의 쥐들은 운동이 끝난후 10분간의 휴식을 한뒤 희생되었는데 이는 운동후 회복기간 동안에 효소 활성도의 time course를 보기 위함이었다. 실험결과 두시간 동안의 운동은 간 조직과 심장 조직 모두에 있어 branched-chain α-keto acid dehydrogenase의 기초 활성도를 현저하게 증가시켰으며 최대 활성도에는 영향을 미치지 않았다. 따라서 최대 활성도에 대한 기초 활성도의 백분율로 나타내는 활성상태(activity state)는 2시간의 운동에 의하여 유의하게 증가되었다. 운동후 10분간의 휴식을 취한 쥐에서는 심장 조직의 효소 활성도가 현저히 저하된 반면 간 조직에서는 운동으로 인하여 증가되었던 효소 활성도가 그대로 남아 있었다. 실험 결과를 종합해 볼 때 운동에 의한 branched-chain α-keto acid dehydrogenase의 활성상태의 증가효과는 효소 자체의 생합성이기 보다는 효소 활성도의 증가로 인한 것임을 알 수 있었다.