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BRIEF COMMUNICATION

Profile of Plasma Amino Acid Levels in Rats Exposed to Acute Hypoxic Hypoxia

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Abstract The effect of acute hypoxic hypoxia on the profile of plasma amino acids in rats was studied and compared to that resulting from acute liver injury induced by giving carbon tetrachloride. In hypoxic rats exposed to 45% air in N_2 for 5 h, the concentrations of branched chain amino acids, including valine, leucine and isoleucine, and aromatic amino acids such as phenylalanine and tyrosine were significantly increased as compared to those in normoxic rats. The ratio of branched-chain to aromatic amino acids (Fischer's ratio) was significantly decreased. The levels of arginine and citrulline, which are related to the urea cycle, were also depressed. Furthermore, plasma proline level was reduced in hypoxic rats. The activities of plasma marker enzymes for tissue damage remained unchanged during hypoxia, indicating that tissue injury was not induced by exposure to hypoxic conditions. We suggest that the characteristic profile of plasma amino acids and the Fischer ratio are valuable tools for understanding the pathology of acute hypoxia in the absence of systemic tissue damage.

Keywords Hypoxia · Fischer's ratio · Branched-chain amino acids · Aromatic amino acids · Amino acids · Rat

Introduction

Hypoxia is defined as oxygen deficiency at the tissue level. Hypoxic hypoxia is the form of this condition most frequently observed clinically and is a complication of various respiratory system diseases. Exposure of human and laboratory animals to hypoxia results in metabolic failure [1–3].

The determination of plasma free amino acid levels is important when studying protein and amino acid metabolism. The branched chain amino acids (BCAAs), which include leucine, isoleucine, and valine, are readily metabolized in the muscle [4], whilst, aromatic amino acids (AAAs), which include phenylalanine and tyrosine, are degraded in the liver. The plasma level of this latter group can be an indicator of liver diseases [5].

In the present paper, we compared plasma amino acid profiles in rats exposed to either acute hypoxia or liver injury from acute carbon tetrachloride (CCl_4) administration.

Materials and Methods

Exposure of Rats to Hypoxia

Male Sprague–Dawley rats (8 weeks old) were obtained from Japan SLC Co., Inc. (Nishiku, Hamamatsu, Shizuoka, Japan). The animals were caged in 50 l chambers into which a gas mixture (9.5% oxygen) composed of 45% air and 55% N_2 was delivered continuously at a flow rate of about 20 l/min. Control rats were exposed to normoxia under the same conditions except that the gas mixture was replaced with 100% air (21% oxygen). After 5 h of exposure, rats were lightly anesthetized with diethyl ether and blood samples were drawn from the abdominal aorta into tubes containing heparin. These samples were centrifuged at 3,000 rpm for 5 min and the plasma obtained was used for the amino acid and enzyme assays.

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Liver Injury by CCl_4

Rats were deprived of food for 12 h after which CCl_4 (50% w/v olive oil) was injected intraperitoneally at a dose of 0.5 g/kg of body weight. Control rats were treated with olive oil only. After 24 h, plasma was obtained using the same approach as described for the hypoxic animals.

Determination of Amino Acid and Enzyme Concentrations

Plasma amino acid concentrations were assayed using the HPLC method of SRL Co., Inc. (Hachioji, Tokyo, Japan), which provides a clinical laboratory testing service [6]. Plasma lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were assayed as described in earlier reports [7].

Calculation of Fischer's Ratio

Fischer's ratio was calculated as the sum of the plasma valine, leucine and isoleucine (BCAAs) levels divided by the sum of the phenylalanine and tyrosine (AAAs) levels [8].

Ethics

This study was performed under the control of the institutional ethics committee, in accordance with the guidelines on animal experimentation at the Faculty of Health Sciences, Kyorin University, Japan.

Results

To assess the extent of cell damage during hypoxia, the activities of plasma ALT, AST and LDH were determined. The activities of these enzymes remained unchanged (Table 1), indicating that the cells did not sustain significant injury as a result of 5 h of hypoxia.

Plasma amino acid levels after exposure to hypoxia or normoxia are summarized in Table 2. Levels of both the BCAA and AAA groups of amino acids were significantly elevated by hypoxia whilst, conversely, citrulline and arginine, which are intermediates in the urea cycle, were significantly decreased by this treatment. Exposure of rats to hypoxia caused a significant decrease in the Fischer's ratio as the rise in the levels of the plasma AAAs was greater than that of the BCAAs. Furthermore, the level of plasma proline decreases after exposure of rats to hypoxia.

The effects of CCl_4 administration on the levels of plasma amino acids were also studied. As summarized in Table 3, activities of plasma ALT, AST and LDH increased markedly in rats treated with CCl_4, indicating that the liver parenchymal cells had been seriously damaged. Most plasma amino acids other than arginine were significantly increased by this treatment (Table 4). Arginine, in contrast, decreased to levels below that detectable by the HPLC method used for the amino acid assay. The

Table 1 Activities of ALT, AST and LDH in the plasma of rats exposed to hypoxia

	ALT (U/L)	AST (U/L)	LDH (U/L)
Normoxia	37.4 ± 9.1	54.1 ± 14.7	183.7 ± 47.9
Hypoxia	41.5 ± 10.1	60.5 ± 17.8	193.6 ± 54.5

Rats were exposed to 45 or 100% air for 5 h. Five rats were used in each of the two groups

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (compared to the values of rats exposed to normoxia)

Table 2 Plasma amino acid concentration and Fischer's ratio in rats exposed to hypoxia

	Normoxia (nmol/ml)	Hypoxia (nmol/ml)
Aspartic acid	27.2 ± 7.7	20.1 ± 3.9
Hydroxyproline	54.8 ± 14.6	48.3 ± 8.1
Threonine	273.1 ± 34.8	235.8 ± 24.9
Serine	169.3 ± 8.0	175.4 ± 25.6
Asparagine	59.0 ± 3.49	51.9 ± 6.5
Glutamic acid	147.3 ± 43.6	119.9 ± 24.6
Glutamine	730.6 ± 22.3	671.4 ± 69.1
Proline	163.0 ± 7.95	129.8 ± 16.4**
Glycine	230.4 ± 19.5	216.4 ± 34.1
Alanine	419.3 ± 35.2	422.5 ± 102.0
Citrulline	91.0 ± 9.1	68.0 ± 5.2**
Valine	205.3 ± 25.4	265.1 ± 24.6**
Methionine	60.1 ± 7.4	62.1 ± 6.7
Isoleucine	99.2 ± 12.3	145.5 ± 10.4***
Leucine	173.9 ± 23.2	224.1 ± 16.0**
Tyrosine	81.6 ± 20.9	149.3 ± 52.9*
Phenylalanine	72.3 ± 6.0	135.8 ± 35.2***
Histidine	68.8 ± 2.6	66.3 ± 5.6
Tryptophan	123.2 ± 16.0	127.4 ± 7.3
Ornithine	69.8 ± 6.8	66.8 ± 9.1
Lysine	403.4 ± 95.9	405.5 ± 87.3
Arginine	204.6 ± 20.2	119.5 ± 48.6**
BCAA	478.5 ± 60.7	634.6 ± 47.9**
AAA	154.0 ± 24.7	285.2 ± 108.0
Fischer's ratio	3.13 ± 0.34	2.38 ± 0.54*

Rats were exposed to 45 or 100% air for 5 h. Five rats were used in each of the two groups

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (compared to the values of rats exposed to normoxia)

Table 3 Activities of ALT, AST and LDH in the plasma of rats administrated with CCl_4

	ALT (U/L)	AST (U/L)	LDH (U/L)
Control	37.9 ± 5.2	50.1 ± 12.5	197.3 ± 46.2
CCl_4	7056.5 ± 302.1***	5753.3 ± 939.9***	18176.8 ± 2766.7***

Eight rats were used in the control treatment and nine were administered with CCl_4

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (compared to the values of rats that were not treated with CCl_4)

Table 4 Plasma amino acid concentration and Fischer's ratio in rats administrated with CCl_4

	Control (nmol/ml)	CCl_4 administration (nmol/ml)
Aspartic acid	15.2 ± 3.8	89.8 ± 45.9**
Hydroxyproline	52.8 ± 3.0	114.0 ± 22.3***
Threonine	170.4 ± 14.9	725.0 ± 276.8***
Serine	199.5 ± 13.4	902.7 ± 344.1***
Asparagine	53.0 ± 2.1	180.7 ± 75.2***
Glutamic acid	91.0 ± 9.4	410.8 ± 197.1**
Glutamine	543.0 ± 16.2	2449.7 ± 1069.4***
Proline	155.1 ± 5.8	446.6 ± 210.3**
Glycine	204.9 ± 13.1	1267.4 ± 624.3***
Alanine	514.1 ± 22.9	2752.2 ± 1618.8**
Citrulline	58.7 ± 4.4	110.3 ± 31.7**
Valine	173.4 ± 11.7	495.3 ± 174.2***
Methionine	54.4 ± 3.6	391.2 ± 249.4**
Isoleucine	70.4 ± 5.4	202.4 ± 71.8***
Leucine	125.9 ± 9.7	379.5 ± 137.2***
Tyrosine	64.9 ± 5.1	343.8 ± 226.0**
Phenylalanine	52.2 ± 3.8	166.8 ± 70.1**
Histidine	70.0 ± 3.9	145.7 ± 91.9*
Tryptophan	94.5 ± 7.7	135.8 ± 16.4***
Ornithine	51.7 ± 5.5	620.4 ± 252.9***
Lysine	339.7 ± 19.2	2599.0 ± 1490.5**
Arginine	147.2 ± 12.1	Not detected
BCAA	369.7 ± 26.4	1077.4 ± 383.1***
AAA	117.2 ± 7.2	510.6 ± 295.5**
Fischer's ratio	3.15 ± 0.19	2.36 ± 0.51**

Eight rats were used in the control treatment and nine were administered with CCl_4

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (compared to the values of rats that were not treated with CCl_4)

treatment of rats with CCl_4 caused a significant decrease in the Fischer's ratio.

Discussion

Tissue hypoxia occurs when tissues experience a low oxygen availability. The particular situation of hypoxic hypoxia results from high altitude climbing in otherwise healthy individuals and from a variety of diseases of the respiratory system. These conditions lead to a systemic deficiency in O_2 that causes metabolic histological changes. Almost all oxygen captured by the lungs is utilized for ATP synthesis by oxidizing glucose and fatty acids in the mitochondria of cells. As a consequence, inadequate ATP is produced by hypoxic cells, leading to cellular metabolic dysfunction.

We studied the effect of acute hypoxic hypoxia on the levels of plasma amino acids in a rat model and found that the levels of both the BCAAs and AAAs were increased by this treatment. BCAAs constitute much of the mass of muscle where they are used for the production of ATP and protein synthesis after incorporation into the tissue [4]. However, oxygen deficiency prevents the use of BCAAs as the mitochondrial electron transfer system is unable to operate smoothly. The concentration of BCAAs in the plasma increases as a consequence of this. In contrast, the AAA group, which includes tyrosine and phenylalanine, is mainly metabolized by the liver. Thus, an elevation in the levels of AAAs is likely to result from the attenuation of the metabolic capacity of the liver caused by exposure to hypoxic conditions.

The blood amino acid profile associated with hepatic cirrhosis is characterized by an increase in the levels of AAAs and a decrease in the levels of BCAAs [9]. The main cause of the decrease in plasma BCAAs is the heightened uptake of BCAAs by muscular tissue during the chronic malnutrition associated with this condition. Meanwhile, the increase in plasma AAAs depends on a decline in the metabolic capacity of the liver. Therefore, a decrease in the Fischer ratio (BCAAs/AAAs ratio) is used as an indicator of liver function during hepatic cirrhosis. In the current study, acute hepatic injury induced by a single administration of CCl_4 resulted in an elevation in both the levels of BCAAs and AAAs and significant decrease in the Fischer ratio. Holecek et al. [10] reported that the Fischer ratio remained unaffected by acute liver damage, in contrast to the reported response to liver cirrhosis. The reason for difference between their and our results may be attributed to the severity of liver damage, as judged by amino acid levels and activities of marker enzymes in liver injury. In the present study, a lower ratio was also observed in rats exposed to acute hypoxic hypoxia in comparison to

normoxic rats. Therefore, Fischer's ratio is useful in evaluating the effects of acute hypoxia without systemic tissue damage.

Administration of CCl_4 to rats resulted in a fall in plasma arginine to a level below that detectable, while the level of ornithine increased significantly. Marked increases in AST, ALT and LDH as markers of hepatic injury indicate that serious and acute liver damage occurred. These observations suggest that a large amount of the arginase contained in the liver is likely to have leaked out into the circulating blood and led to the conversion of arginine to ornithine [10, 11]. Exposure to hypoxia, however, resulted in no change in the levels of these marker enzymes compared to normoxic controls, suggesting that hepatic injury had not occurred under the hypoxic conditions used. In hypoxic rats, the plasma arginine level decreased slightly, whilst citrulline showed a characteristically significant decline. These latter changes are indicative that the normal functioning of the urea cycle was disturbed by exposure to hypoxia. The reaction in the hepatic urea cycle catalyzed by mitochondrial carbamoyl-phosphate synthetase is absolutely dependent upon ATP. Thus, the depletion of carbamoyl-phosphate resulting from ATP depletion after hypoxic exposure in turn causes a decrease in plasma citrulline that is formed by ornithine carbamoyl transferase. In hypoxic rats, we found a decrease in plasma proline level. Proline synthesis involves a four step process starting with glutamate. The first reaction requires γ-glutamyl kinase that depends on ATP, leading to depression of proline production. The reduction in the citrulline and proline levels is typical of acute hypoxic hypoxia, although acute liver damage induced by CCl_4 injection actually raised the level of this amino acid.

In conclusion, we propose that the characteristic profiles of plasma amino acids and the Fischer ratio are valuable tools for assessing the pathology associated with acute hypoxic hypoxia.

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