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Alterations in mitochondrial branched-chain amino acid metabolism in brain in acute hyperammonemic states

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Production of $^{14}CO_2$ and [^{14}C]branched-chain keto acids (BCKA) was determined from [U-^{14}C]branched-chain amino acids along with the activities of branched-chain amino acid transaminase (BCAA-T) and branched-chain keto acid dehydrogenase (BCKA-DH) in mitochondria isolated from the cerebral cortex of normal and hyperammonemic rats. Results indicated that the production of CO_2, but not of keto acids, was suppressed while the activities of BCAA-T and BCKA-DH were not adversely affected in the mitochondria of hyperammonemic rats. Suppression in the oxidation of BCAA in hyperammonemic states was found to be due to increased efflux of BCKA from mitochondria.

Glutamine synthesis is the major mechanism by which ammonia is disposed in the brain [5]. This process has been implicated in the pathophysiology of the cerebral dysfunction in hyperammonemic states. However, there appears to be a controversy on the nature of the reaction that supplies glutamate for the synthesis of glutamine under these conditions. Berl [3] proposed that the glutamate produced in the reaction mediated by glutamate dehydrogenase in the small pool of glutamate (presumably localized in the astrocytes) might serve as the precursor for the synthesis of glutamine, while Benjamin and Quastel [2] proposed that the glutamate released by the large compartment (presumably neuronal) might be the precursor for the synthesis of glutamine. However, insignificant incorporation of ^{13}N- and ^{15}N-labeled ammonia into the α-amino nitrogen of glutamate and glutamine negated the earlier concept [5, 18]. The amount of glutamate released from neurons might not be adequate to support the synthesis of glutamine in hyperammonemic conditions. Hence, it was proposed that branched-chain amino acids (BCAA; leucine, isoleucine, and valine) might provide glutamate for the synthesis of glutamine [5, 6]. It was also shown that glutamate produced from these amino acids might account for one third of the total glutamate present in the brain [17].

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Very few reports are available on the cerebral metabolism of BCAA in hyperammonemic states. Shiota [16] reported enhanced production of $^{14}CO_2$ from [1-^{14}C]leucine in brain in hyperammonemic states. However, Murthy and Hertz [11, 12] reported a suppression in the production of $^{14}CO_2$ from [U-^{14}C]BCAA in the primary cultures of astrocytes exposed to pathophysiological concentrations of ammonium chloride. This is in contrast to the reported elevation in the activities of branched-chain amino acid transaminase in different brain regions of hyperammonemic animals [8, 9].

Presently, we report changes in the production of $^{14}CO_2$ and [^{14}C]branched-chain keto acids (BCKA) from [U-^{14}C]BCAA, along with changes in the activities of BCAA-transaminase (BCAA-T) and BCKA dehydrogenase (BCKA-DH) in the non-synaptic mitochondria (referred hereafter as mitochondria) isolated from the cerebral cortex of hyperammonemic rats. We observed a suppression in the oxidation of BCAA in hyperammonemic states. However, there was no decrease in the activities of BCAA-T and an elevation in BCKA-DH activity under these conditions. It was observed that a major portion of the BCKA, produced in the transamination reaction, was not oxidized but transported out of the mitochondria and this efflux of BCKA was enhanced in hyperammonemic states resulting in a decreased production of $^{14}CO_2$ from [U-^{14}C]BCAA.

Induction of hyperammonemia in adult Wistar rats (150–200 g), isolation of mitochondria from the cerebral

cortex of these animals, assay methods for marker enzymes for mitochondria and BCAA-T, and determination of blood and brain (frozen in liquid nitrogen) ammonia content and mitochondrial protein content have been described earlier [8, 14]. BCKA-DH activity was determined by the method of Connelly et al. [4]. Production of $^{14}CO_2$ from [U-^{14}C]BCAA was determined by the method of Rao and Murthy [13] except that labeled glutamate was replaced with [U-^{14}C]BCAA (100 μM; 0.5 μCi) in the incubation medium.

To determine the production of [^{14}C]BCKA, mitochondria were incubated with [U-^{14}C]BCAA under conditions identical to those of CO_2 production. After 30 min of incubation at 37°C, incubation medium along with mitochondria was divided into two aliquots. To one of these, 1 ml of 2,4-dinitrophenylhydrazine (0.25% in 1 N HCl) was added while the other was centrifuged at 10,000 × g for 5 min at 2°C. Dinitrophenylhydrazine was added separately to the medium and pellet. After 10 min, hydrazone was extracted into 1 ml of cyclohexane. The cyclohexane layer was concentrated and chromatographed on Whatman No. 1 paper using n-butanol:acetic acid:water (65:15:25 v/v). Radioactivity in the spots corresponding to authentic keto acid standards was determined. Under these conditions, it was observed that about 95% of the radioactivity present in the cyclohexane layer was that of the keto acid. Statistical significance of the results was analyzed by Student's t-test. Results are presented in Table I.

Blood and brain ammonia levels in the hyperammonemic animals were similar to those reported earlier [14]. Under these conditions, there was a 6- and 26-fold increase in the contents of ammonia in the blood and brain, respectively. Purity of mitochondrial preparations isolated from normal and hyperammonemic animals, as determined by the activities of marker enzymes, was similar to those reported earlier [14].

In the mitochondria of normal animals, all the three

TABLE I

ACTIVITIES OF BRANCHED-CHAIN AMINO ACID TRANSAMINASE, BRANCHED-CHAIN KETO ACID DEHYDROGENASE, PRODUCTION OF $^{14}CO_2$ AND [^{14}C]BRANCHED-CHAIN KETO ACIDS FROM [U-^{14}C]BRANCHED CHAIN AMINO ACIDS IN THE MITOCHONDRIA OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

All the values are mean ± S.D. Numbers in parentheses indicate the number of experiments. a: μmol of keto acid formed/mg protein/h; b: pmol of CO_2 produced/mg protein/h; c: μmol of ferricyanide reduced/mg protein/h; d: pmol of keto acid/mg protein/h. KIC, α-ketoisocaproic acid; KIV, α-ketoisovaleric acid; KMV, α-keto-β-methylvaleric acid.

	Leucine	Valine	Isoleucine
BCAA-T activity[a]			
Normal	2.3 ± 0.4 (5)	5.6 ± 0.9 (4)	5.5 ± 1.2 (4)
Hyperammonemic	7.2 ± 1.6 (5)**	4.2 ± 0.8 (6)	5.4 ± 1.2 (5)
CO_2 production[b]			
Normal	630 ± 60 (5)	570 ± (5)	980 ± 100 (5)
Hyperammonemic	420 ± 30 (5)**	970 ± 250 (5)$^{+}$	360 ± 90 (6)**
	KIC	KIV	KMV
BCKA-DH activity[c]			
Normal	2.5 ± 0.6 (7)	3.4 ± 0.8 (9)	2.3 ± 0.7 (6)
Hyperammonemic	6.9 ± 1.2 (6)*	5.8 ± 1.0 (5)*	4.2 ± 1.2 (5)*
BCKA content -- total[d]			
Normal	1680 ± 500 (5)	1800 ± 400 (6)	2200 ± 300 (8)
Hyperammonemic	1700 ± 600 (5)	2200 ± 600 (6)	2300 ± 500 (5)
BCKA content — pellet[d]			
Normal	120 ± 40 (3)	190 ± 30 (3)	110 ± 30 (4)
Hyperammonemic	140 ± 40 (3)	160 ± 30 (3)	680 ± 110 (4)**
BCKA content — supernatant[d]			
Normal	1500 ± 175 (3)	1810 ± 600 (3)	1700 ± 500 (8)
Hyperammonemic	2800 ± 500 (3)$^{++}$	2700 ± 600 (3)$^{++}$	2850 ± 400 (5)$^{+}$

$^{+}P < 0.01$; $^{++}P < 0.025$; $^{*}P < 0.02$; $^{**}P < 0.001$.

BCAA were transaminated and the activity of this enzyme with isoleucine and valine was higher than with leucine. Administration of ammonium acetate resulted in an elevation in the activity of this enzyme with leucine as substrate. However, with isoleucine and valine as substrates, there were no statistically significant changes.

Activity of BCKA-DH with all the three BCKA was found to be similar in the mitochondria of normoammonemic animals. Administration of ammonium acetate resulted in an elevation in the activity of this enzyme. The magnitude of elevation was observed to be highest with α-ketoisocaproic acid (KIC) as substrate.

Cortical mitochondria of normal and hyperammonemic animals were capable of oxidizing all the three BCAA. In the mitochondria of normal animals, production of $^{14}CO_2$ was higher with [U-^{14}C]isoleucine than with leucine and valine. Rate of production of $^{14}CO_2$ from the latter two BCAA was observed to be similar. Administration of ammonium acetate was found to have different effects on the production of $^{14}CO_2$ from the three BCAA. The rate of production of $^{14}CO_2$ from [U-^{14}C]leucine and isoleucine was suppressed while that from [U-^{14}C]valine was enhanced. The magnitude of suppression was greater with isoleucine than with leucine.

Total content of the BCKA in the mitochondria of normal and hyperammonemic animals was observed to be higher than the rate of production of $^{14}CO_2$. Less than 10% of the total BCKA produced in the mitochondria was retained in the organelle while a large portion of BCKA was found to be in the supernatant.

There were no statistically significant differences in the total content of BCKA in the mitochondria of hyperammonemic animals when compared to that of the normal animals. Similarly, there were no significant differences in the contents of either KIC or α-ketoisovaleric acid (KIV) in the mitochondrial pellet of normal and hyperammonemic animals. However, α-keto-β-methylvaleric acid content was found to be elevated in the mitochondria of the hyperammonemic animals. In contrast to this, contents of all the three BCKA were elevated in the supernatant prepared after the incubation of mitochondria of hyperammonemic animals with labeled BCAA. The magnitude of elevation was found to be the same with all the three BCKA.

Results of the present study indicated that mitochondrial BCAA-T was capable of utilizing all the three BCAA resulting in the production of glutamate and respective BCKA. Glutamate, so formed in the mitochondria, might be converted to α-ketoglutarate either by the action of other aminotransferases (aspartate and alanine aminotransferases) or by the action of glutamate dehydrogenase. The BCKA formed in BCAA-T reaction would serve as substrates for BCKA-DH and the ketoacyl CoA formed in this reaction would be metabolized either to acetyl CoA (leucine and isoleucine) or to succinyl CoA (isoleucine and valine) which are ultimately oxidized in the citric acid cycle. Higher rates of isoleucine oxidation might be due to the fact that the metabolism of this amino acid results in the production of both acetyl and succinyl CoA.

Measured amounts of BCKA in normal animals indicated that only a small portion of the total BCKA was utilized for oxidation while a large portion was transported out of the mitochondria. This process restricts oxidation, thus the loss, of carbon skeleton of BCAA. Following the transport, this keto acid may be reutilized in the same or in a different cell. As astrocytes are supposed to be involved in the metabolism of BCAA, the BCKA may be transaminated with glutamine and a recent report on the incorporation of ^{15}N-nitrogen of glutamine into leucine supports this concept [19].

Lack of changes in BCAA-T activity with isoleucine and valine and an elevation in the activity of this enzyme with leucine as substrate suggested that transamination of BCAA, thus rate of formation of BCKA, might be unaffected in brain in hyperammonemic states. Elevation in the activity of BCKA-DH under these conditions was suggestive of an increase in the oxidative metabolism of BCKA. The observed decrease in CO_2 production from BCAA in hyperammonemic states was in agreement with results in the primary cultures of astrocytes exposed to pathophysiological concentrations of ammonium ions [11, 12]. The anomaly in the effects of ammonia on CO_2 production and BCKA-DH activity could be due to decreased operational rates of citric acid and/or increased efflux of BCKA from the mitochondria which makes them unavailable for the oxidative processes. The earlier possibility may be ruled out as the available evidences indicate no adverse effects of acute hyperammonemic states on the operational rates of citric acid cycle in brain [1, 7, 14, 15, 20]. Lack of changes in the total (tissue + medium) BCKA content and an elevation in the content of these keto acids in the medium indicated that the outflow of BCKA from cerebral mitochondria was enhanced in acute hyperammonemic states. It is suggested that (i) increased transamination of BCAA in hyperammonemic states provides the glutamate required for the synthesis of glutamine; (ii) increased transport of BCKA from the mitochondria prevents the loss of carbon skeleton of BCAA, facilitates their re-utilization and prevents the BCKA from exerting their inhibitory effects on pyruvate oxidation in brain [10].

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