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Activities of Pyruvate Dehydrogenase, Enzymes of Citric Acid Cycle, and Aminotransferases in the Subcellular Fractions of Cerebral Cortex in Normal and Hyperammonemic Rats

L. Ratnakumari[1] and Ch. R. K. Murthy[1,2]

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Activity levels of pyruvate dehydrogenase, enzymes of citric acid cycle, aspartate and alanine aminotransferases were estimated in mitochondria, synaptosomes and cytosol isolated from brains of normal rats and those injected with acute and subacute doses of ammonium acetate. In mitochondria isolated from animals treated with acute dose of ammonium acetate, there was an elevation in the activities of pyruvate, isocitrate and succinate dehydrogenases while the activities of malate dehydrogenase (malate→oxaloacetate), aspartate and alanine aminotransferases were suppressed. In subacute conditions a similar profile of change was noticed excepting that there was an elevation in the activity of α-ketoglutarate dehydrogenase in mitochondria. In the synaptosomes isolated from animals administered with acute dose of ammonium acetate, there was an increase in the activities of pyruvate, isocitrate, α-ketoglutarate and succinate dehydrogenases while the changes in the activities of malate dehydrogenase, aspartate and alanine amino transferases were suppressed. In the subacute toxicity similar changes were observed in this fraction except that the activity of malate dehydrogenase (oxaloacetate→malate) was enhanced. In the cytosol, pyruvate dehydrogenase and other enzymes of citric acid cycle except malate dehydrogenase were enhanced in both acute and subacute ammonia toxicity though their activities are lesser than that of mitochondria. In this fraction malate dehydrogenase (oxaloacetate→malate) was enhanced while activities of malate dehydrogenase (malate→oxaloacetate), aspartate and alanine aminotransferases were suppressed in both the conditions. Based on these results it is concluded that the decreased activities of malate dehydrogenase (malate→oxaloacetate) in mitochondria and of aspartate aminotransferase in mitochondria and cytosol may be responsible for the disruption of malate-aspartate shuttle in hyperammonemic state. Possible existence of a small vulnerable population of mitochondria in brain which might degenerate and liberate their contents into cytosol in hyperammonemic states is also suggested.

KEY WORDS: Citric acid cycle enzymes; hyperammonemia; cytosol; mitochondria; synaptosomes.

INTRODUCTION

Derangement in the cerebral energy metabolism was proposed to be one of the many mechanisms by which ammonia exerts toxic effects on the central nervous system. It was hypothesized that removal of α-ketoglutarate

[1] School of Life Sciences, University of Hyderabad, Hyderabad - 500 134, India.

[2] Address reprint request to: Dr. Ch. R. K. Murthy, School of Life Sciences, University of Hyderabad, HYDERABAD 500 134 AP, India.

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from citric acid cycle for the purpose of ammonia detoxification (in the glutamate dehydrogenase reaction), results in the formation of glutamate and the conversion of the latter to glutamine, would adversely affect the cerebral energy stores (1–3). Moreover, by removing cytosolic pool of glutamate for the synthesis of glutamine, ammonia was postulated to interfere with the operation of malate-aspartate shuttle and thereby the transport of reducing equivalents from cytosol to mitochondria (4, 5). Several conflicting reports were made in the past with respect to the depletion of α-ketoglutarate stores while evidences have accumulated which strongly favour the latter concept (6–9). It was observed that not many studies were made in the past on the subcellular distribution and changes in the activities of the enzymes involved in carbohydrate metabolism in hyperammonemia.

Earlier, we reported an elevation in the activities of pyruvate dehydrogenase and enzymes of citric acid cycle, except malate dehydrogenase, in the homogenates prepared from different regions of brains of hyperammonemic rats (10). We have also reported a fall in the activities of aspartate and alanine aminotransferases, malate dehydrogenase and NADP-dependent isocitrate dehydrogenase in these preparations and suggested that transport of reducing equivalents across mitochondria might be affected in hyperammonemic states. As brain has two types of mitochondrial populations i.e., synaptic and non-synaptic and both these have citric acid cycle enzymes, a study with homogenates will not reveal whether the changes in the activities of these enzymes are occurring in the synaptic or non-synpatic mitochondria. Moreover, enzymes involved in the transport of reducing equivalents are present in both cytosol and mitochondria. Hence it becomes essential to localize the changes (suppression) in the activities of the enzymes of malate-aspartate shuttle in the brains of hyperammonemic rats and such an attempt has been made in the present study.

Based on the results obtained in the present study, we suggest that (i) suppression of malate-aspartate shuttle may be due to decreased malate dehydrogenase activity in the direction of malate formation in the mitochondria (ii) there is a small population of mitochondria which are vulnerable to patho-physiological ammonia concentrations which degenerate/rupture and liberate their contents into the cytosol and (iii) the apparent increase in citric acid cycle enzymes observed in homogenates may be due to release of these enzymes into cytosol and loss of regulatory control over these enzymes in an altered subcellular environment.

EXPERIMENTAL PROCEDURE

Adult albino rats of Wistar strain of 250–300 gms. body weight were maintained in groups of 6–8 per cage under natural light-dark cycles at a constant temperature. Food and water were provided ad libitum. These animals were divided into three groups with ten animals each and two animals were used for each experiment. Animals in group I were administered intraperitoneally with 0.35 mmol of ammonium acetate/100 g body weights (subacute experiments) and the animals in group II received 2.5 mmol of ammonium acetate per 100 gms. body weight (acute experiments) while group III animals received none and served as controls. Animals in group I and II were sacrificed 25–30 min after the administration of ammonium acetate. Mitochondria, synaptosomes and cytosol were prepared by the method of Cotman (11) as described by Subbalakshmi and Murthy (12). These fractions were frozen overnight and Triton X-100 was added to a final concentration of 0.1% v/v after thawing the preparations.

Protein, present in 20 μl aliquot of subcellular fractions, was determined by the method of Lowry et al. (13). Ammonia content in brains frozen in liquid nitrogen and in the serum was determined as described earlier (12).

Enzyme Assays. Activities of pyruvate dehydrogenase, citrate synthase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, malate dehydrogenase (in the direction of oxaloacetate formation), aspartate and alanine aminotransferases were assayed as described earlier (10). Malate dehydrogenase activity, (in the direction of malate formation) was assayed as suggested by Yoshida (14). Lactate and succinate dehydrogenases were assayed by the methods of Bergmeyer and Bernt and Nandakumar et al. (15, 16) respectively. After determining the optimal concentrations of enzyme protein, substrate and cofactors, suitable alterations were made for each enzyme (Table I). Statistical analysis of data was performed by Student's *t* test.

RESULTS

Rats administered with subacute dose of ammonium acetate (group I) showed no convulsions even upto 10 hours. However, they were sacrificed 25–30 min after the administration of ammonium acetate. This time period was chosen as the animals injected with acute dose entered into convulsions at this time period. Rats injected with acute dose of ammonium acetate (group II) exhibited convulsions in about 25–30 min which was usually the terminal phase. In this group all the animals irrespective of their sex, succumbed to the toxic effects of ammonia at about 45 min after the administration of ammonium acetate. Hence, they were sacrificed during convulsions and used for experimentation. After the administration of ammonium acetate there was an increase in blood and brain ammonia levels (Table II).

In normal animals, activities of pyruvate, isocitrate(NAD^+), α-ketoglutarate and succinate dehydrogenases were higher in mitochondrial fraction than in cytosol or synaptosomes. Activities of these enzymes were found

Table I. Methods for the Assay of Pyruvate Dehydrogenase, Lactate Dehydrogenase and Enzymes of TCA Cycle and Borst Cycle With Subcellular Fractions

Enzyme	Method
Pyruvate dehydrogenase (E.C.1.2.4.1)	K-PO_4 buffer (pH 7.8) 50 mM, NAD 2.5 mM, TPP 0.2 mM, CoA 100 μM, DTT 300 μM, Pyruvate 5 mM, $MgCl_2$ 1 mM, PMS 6.50 μM, INT 300 μM. O.D change at 500 nm. (10)
Citrate synthase (E.C.4.1.3.7)	Tris-HCl buffer (pH 8.0) 96 mM, DTNB 97 nM, OAA 240 μM, acetyl CoA 48 μM. O.D. change at 412 nm. (10)
Isocitrate dehydrogenase (E.C.1.1.1.41)	Tris acetate buffer (pH 7.2) 33mM, NAD 333 μM, ADP 6.7 mM, isocitrate 5.3 mM, $MgCl_2$ 1 mM, PMS 6.50 μM, INT 300 μM. O.D change at 500 nm. (10)
α-Ketoglutarate dehydrogenase (E.C.1.2.4.2)	K-PO_4 buffer (pH 8.0) 50 mM, NAD 2.0 mM, TPP 200 μM, CoA 60 μM, α-ketoglutarate 1.0 mM, $MgCl_2$ 1 mM, PMS 6.50 μM, INT 300 μM. O.D change at 500 nm. (10)
Succinate dehydrogenase (E.C.1.3.99.1)	Tris-PO_4 buffer (pH 7.6) 100 mM, succinate 40 mM, PMS 6.50 μM, INT 4 mM, O.D at 500 nm. (16)
Malate dehydrogenase (E.C.1.1.1.37)	Tris-HCl buffer (pH 8.8) 83 mM, NAD 3.3 mM, malate 3.2 mM, INT 300 μM, PMS 6.50 μM. O.D change at 500 nm (10)
Malate dehydrogenase (E.C.1.1.1.37)	Tris-HCl buffer (pH 8.8) 83 mM, NADH 160 μM, oxaloacetate 3.4 mM. O.D change at 340 nm. (14)
Aspartate aminotransferase (E.C.2.6.1.1)	K-PO_4 buffer (pH 7.4) 80 mM, NADH 520 μM, aspartate 20 mM, α-ketoglutarate 18 mM, MDH 10 μg. O.D. change at 340 nm. (15)
Alanine aminotransferase (E.C.2.6.1.2)	K-PO_4 buffer (pH 7.4) 80 mM, NADH 520 μM, alanine 40 mM, α-ketoglutarate 18 mM, LDH 4 μg. O.D. change at 340 nm. (15)
Lactate dehydrogenase (E.C.1.1.1.27)	K-PO_4 buffer (pH 7.5) 48 mM, pyruvate 0.6 mM, NADH 180 μM. O.D. change at 340 nm. (15)
Lactate dehydrogenase (E.C.1.1.1.27)	K-PO_4 buffer (pH 7.5) 48 mM, lactate 1.2 mM, NAD 333 μM, PMS 6.5 μM, INT 300 μm. O.D. change at 500 nm. (15).

In all the above assays, except succinate dehydrogenase, final volume was 250 μl. In the assay for succinate dehydrogenase, the final volume was 1.0 ml and the assay was colorimetric. The assay mixture for SDH was incubated for 15 min and the reaction was arrested with 2 ml of glacial acetic acid. Colour produced due to the formation of formazan was extracted into 5 ml of toluene. In all the assays, the incubation temperature was 37°C. Corrections were made for non specific activity with suitable blanks. In the spectrophotometric assays changes in absorbance were recorded at 15 sec intervals for 10 minutes and the values in linear kinetic zone were used for calculating enzyme activity. Relationship between formazan formed and NAD^+ reduced was established as earlier (10). Abbreviations: TPP: thiamine pyrophosphate; DTT: dithiothreitol; PMS: phenazine methosulphate; INT: 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium chloride; DTNB: 5-5′dithiobis(2-nitrobenzoic acid); CoA: coenzyme A; MDH: malate dehydrogenase; LDH: lactate dehydrogenase; ADP: adenosine diphosphate; NAD: nicotinamide adenine dinucleotide (oxidized); NADH: nicotinamide adenine dinucleotide (reduced). ( ) indicate the reference to the method.

Table II. Ammonia Levels in Blood and Brains of Normal and Hyperammonemic Rats

Condition	Brain	Blood
Normal	0.45 ± 0.12(3)	0.073 ± 0.01(3)
Subacute	1.40 ± 0.35(3)	1.110 ± 0.16(5)
	$P<0.005$ + 211%	$P<0.001$ + 1420%
Acute	2.64 ± 0.50(3)	1.790 ± 0.08(4)
	$P<0.001$ + 487%	$P<0.001$ + 2352%

Values are expressed in brain as μmol of ammonia/gm·wet wt. and in blood as μmol of ammonia/ml. Number in parenthesis indicates number of experiments.

to be higher in synaptosomes than in cytosol. However, in the case of malate dehydrogenase, the magnitude of difference between different fractions was not as high as with other enzymes. Activity levels of aspartate aminotransferase were observed to be higher in synaptosomal and cytosolic fractions than in mitochondria. An opposite trend was observed in the distribution of alanine aminotransferase (Tables IV - VI).

Effects of Ammonia

Mitochondria (Table IV). Administration of subacute dose of ammonium salts (group I) resulted in a marginal elevation in the activity of pyruvate dehydrogenase without altering those of citrate synthase and isocitrate dehydrogenase. Activities of α-ketoglutarate and succinate dehydrogenases were enhanced under these conditions. In contrast to the above said enzymes, malate dehydrogenase activity, when measured in the direction of oxaloacetate formation, was suppressed in subacute ammonia toxicity. However, in the reverse direction, i.e., in the direction of malate formation, it was unaltered. Activities of aspartate and alanine aminotransferases decreased under these conditions.

Administration of an acute dose of ammonium acetate (group II) resulted in an elevation in the activities of pyruvate, isocitrate and succinate dehydrogenases in the mitochondrial fraction. Under these conditions, activities of cirate synthase and α-ketoglutarate dehydrogenase were unaffected. Changes observed in the activities

of malate dehydrogenase and aspartate and alanine aminotransferases were similar to those observed in subacute toxicity. It is interesting to note that there is a small but statistically significant increase in the mitochondrial protein under these conditions (Table III).

Synaptosomes (Table V). Following the administration of a subacute dose of ammonium acetate, there was an elevation in the activities of pyruvate, isocitrate, α-ketoglutarate and succinate dehydrogenases while that of citrate synthase was unchanged in the synaptosomes. Malate dehydrogenase activity, in the direction of oxaloacetate formation, was suppressed in synaptosomes in subacute ammonia toxicity. However, in the reverse direction, activity of this enzyme was enhanced under these conditions. Activities of both the aminotransferases decreased in the synaptosomes in subacute ammonia toxicity.

Pattern of changes in the activities of pyruvate dehydrogenase, citric acid cycle enzymes and the aspartate and alanine aminotransferases in synaptosomes in acute ammonia toxicity were similar to those observed in the subacute toxicity. Exceptions to this were seen in the activity of malate dehydrogenase in the direction of malate formation which was suppressed in acute ammonia toxicity. Though the activity of alanine aminotransferase was suppressed under these conditions, the change was statistically not significant. Protein content of cortical synaptosomes decreased in acute ammonia toxicity while in subacute toxicity this change was statistically not significant (Table III).

Cytosol (Table VI). Administration of a subacute dose of ammonium acetate resulted in an elevation in the activities of pyruvate dehydrogenase, citrate synthase, isocitrate, α-ketoglutarate and succinate dehydrogenases in the cytosol. Eventhough the magnitude of their increase in cytosol was much higher than that in the other two fractions, activities of these enzymes in the cytosol were lower than that of mitochondria and

Table III. Protein Levels in Subcellular Fractions of Cerebral Cortex in Normal and Ammonium Acetate Injected Rats

Fraction	Normal	Acute	Subacute
Mitochondria	7.0 ± 1.0(21)	9.3 ± 2.7(15) $P<0.005$ +33%	8.0 ± 2.0(9) NS +14%
Synaptosomes	9.0 ± 2.8(16)	7.3 ± 1.2(15) $P<0.05$ −19%	8.4 ± 2.0(9) NS −7%
Cytosol	28.0 ± 4.0(24)	29.9 ± 3.0(20) NS +7%	27.0 ± 4.0(9) NS −4%

Protein: mg/gm·wet wt of tissue. Numbers in parenthesis indicates the number of experiments. Each value is mean ± SD.

Table IV. Effect of Ammonium Acetate on Pyruvate Dehydrogenase and Enzymes of Citric Acid Cycle and of Borst Cycle in the Mitochondria of Rat Cerebral Cortex

Enzyme	Normal	Acute	Subacute
1. PDH	9.66 ± 0.52	15.2 ± 0.37 $P<0.02$ +57%	11.0 ± 1.1 $P<0.05$ +14%
2. CS	65 ± 8	62 ± 11 NS −5%	67 ± 12 NS +3%
3. ICDH (NAD)	35.8 ± 4	43 ± 5 $P<0.05$ +20%	39.5 ± 1.3 NS +10%
4. α−KGDH	22 ± 3.7	26 ± 3.9 NS +18%	32 ± 3.7 $P<0.005$ +45%
5. SDH	2.8 ± 0.43	5.56 ± 0.7 $P<0.001$ +99%	4.94 ± 0.9 $P<0.005$ +76%
6. MDH (MAL→OAA)	536 ± 41	341 ± 24 $P<0.001$ −36%	365 ± 15 $P<0.001$ −32%
7. MDH (OAA→MAL)	472 ± 43	508 ± 8 NS +8%	464 ± 38 NS −2%
8. AAT	150 ± 15	82 ± 9 $P<0.001$ −45%	99 ± 7.6 $P<0.001$ −34%
9. AlAT	23 ± 3.7	2.7 ± 0.23 $P<0.001$ −88%	8.7 ± 1.8 $P<0.001$ −62%

Activity is expressed as Mean ± SD.
PDH: pyruvate dehydrogenase; CS: citrate synthetase; ICDH: isocitrate dehydrogenase; α-KGDH: α-ketoglutarate dehydrogenase; SDH: succinate dehydrogenase; MDH: malate dehydrogenase; AAT: aspartate aminotransferase; AlAT: alanine aminotransferase.
Activity units for PDH, ICDH, α-KGDH, MDH(NAD) are μmol of NAD reduced/mg protein/hr and for CS is μmol of citrate formed/mg protein/hr, SDH is μmol of succinate oxidized/mg protein/hr and for MDH(NADH), AAT and AlAT is μmol of NADH oxidized/mg protein/hr No. of experiments are 5. For each experiment two animals were used.

Table V. Effect of Ammonium Acetate on Pyruvate Dehydrogenase and Enzymes of Citric Acid Cycle and of Borst Cycle in the Synaptosomes of Rat Cerebral Cortex

Enzyme	Normal	Acute	Subacute
1. PDH	3.42 ± 0.33	13.2 ± 2.6 $P<0.001$ +285%	8.7 ± 0.77 $P<0.001$ +154%
2. CS	32 ± 4	32 ± 2 NS	37 ± 8 NS +16%
3. ICDH (NAD)	11.4 ± 1.72	16.2 ± 3.3 $P<0.025$ +42%	15.7 ± 0.99 $P<0.005$ +32%
4. α-KGDH	19.5 ± 1.99	24 ± 3.3 $P<0.05$ +23%	25 ± 1.62 $P<0.005$ +28%
5. SDH	0.53 ± 0.06	0.92 ± 0.15 $P<0.005$ +74%	0.73 ± 0.016 $P<0.001$ +38%
6. MDH (MAL→OAA)	388 ± 33	251 ± 21 $P<0.005$ −35%	285 ± 24 $P<0.005$ −27%
7. MDH (OAA→MAL)	462 ± 35	383 ± 25 $P<0.005$ −17%	556 ± 28 $P<0.005$ +20%
8. AAT	192 ± 8.3	84 ± 12 $P<0.001$ −56%	108 ± 13 $P<0.001$ −44%
9. AlAT	5.2 ± 0.7	4.3 ± 0.53 NS −18%	3.8 ± 0.22 $P<0.005$ −27%

Legend as in Table IV.

synaptosomes. As observed in the other two fractions, malate dehydrogenase activity in the direction of malate to oxaloacetate was suppressed. However, activity of this enzyme in reverse direction was elevated. Activities of both the aminotransferases were suppressed in this subcellular fraction following the administration of subacute dose of ammonium acetate.

Administration of acute dose of ammonium acetate brought about changes in the activities of these enzymes which are similar to those described above. In the case of pyruvate dehydrogenase, citrate synthase, isocitrate, α-ketoglutarate and succinate dehydrogenases and alanine aminotransferase, the magnitude of change under these conditions was higher than that of subacute toxicity.

DISCUSSION

In studies dealing with subcellular fractions, it is customary to establish the purity of the fractions by determining the marker enzymes. However, caution must be exerted in situations where the activities of marker enzymes are also altered in the experimental condition (17). In the present case, activities of the markers (lactate dehydrogenase and succinate dehydrogenase for cytosol and mitochondria respectively) were also altered substantially in the hyperammonemic state (Table VII). Hence, the relative activities of the marker enzymes were taken into consideration. Changes in the ratio of succinate dehydrogenase activity between mitochondria and synaptosomes were statistically not significant indicating that there were no alterations in the purity of these preparations. However, the cytosol/mitochondria ratio of lactate dehydrogenase was altered only in the subacute condition. A statistically significant increase in the ratio for succinate dehydrogenase in these two fractions was observed in hyperammonemic states. Though these results indicated a contamination of mitochondria with cytosol, this is difficult to comprehend as the buoyant densities of these two fractions are different and they are separated at an early stage of preparation. Hence, the changes in these ratios are due to drug induced changes in the activities of lactate and succinate dehydrogenases rather than contamination of fractions.

Increased activity of pyruvate dehydrogenase observed presently in the cortical mitochondria isolated from the brains of hyperammonemic rats is in agreement with our earlier reports in homogenates (10). Such an increase in the activity of this enzyme might permit channelling of more pyruvate into citric acid cycle. However, lack of change in the activities of citrate synthase and isocitrate dehydrogenase in subacute conditions and citrate synthase and α-ketoglutarate dehydrogenase in acute states might limit the flow of carbons through this cycle. Suppression of malate dehydrogenase activity in the direction of oxaloacetate production lowers the formation of oxaloacetate and results in the accumulation of malate. A fall in the production of oxaloacetate would affect the rate of synthesis of citrate. Moreover, this would

Table VI. Effect of Ammonium Acetate on Pyruvate Dehydrogenase and Enzymes of Citric Acid Cycle and of Borst Cycle in the Cytosol of Rat Cerebral Cortex

Enzyme	Normal	Acute	Subacute
1. PDH	1.036 ± 0.18	7.2 ± 0.52 $P<0.001$ +595%	3.46 ± 0.5 $P<0.001$ +234%
2. CS	0.8 ± 0.05	1.8 ± 0.2 $P<0.001$ +125%	1.5 ± 0.4 $P<0.001$ +88%
3. ICDH (NAD)	1.5 ± 0.47	2.9 ± 0.49 $P<0.005$ +93%	2.2 ± 0.32 $P<0.025$ +47%
4. α-KGDH	3.6 ± 0.54	11.4 ± 2.2 $P<0.001$ +220%	5.5 ± 0.55 $P<0.001$ +54%
5. SDH	0.04 ± 0.002	0.33 ± 0.042 $P<0.001$ +725%	0.197 ± 0.018 $P<0.001$ +393%
6. MDH (MAL→OAA)	392 ± 66	228 ± 24 $P<0.001$ −42%	240 ± 22 $P<0.005$ −39%
7. MDH (OAA→MAL)	576 ± 68	719 ± 27 $P<0.005$ +25%	755 ± 69 $P<0.005$ +31%
8. AAT	129 ± 13	71 ± 6 $P<0.001$ −45%	68 ± 7.6 $P<0.001$ −47%
9. A1AT	12 ± 1.8	7.8 ± 0.76 $P<0.001$ −36%	5.98 ± 0.78 $P<0.001$ −52%

Legend as in Table IV.

also limit the amount of oxaloacetate available for transamination of glutamate, which together with the suppression of aspartate aminotransferase, would lower the production of aspartate in mitochondria. As mitochondrial aspartate is exchanged for cytosolic glutamate during the operation of malate-aspartate shuttle, reduction in aspartate levels would affect the operation of this shuttle. The reported fall in aspartate and increase in malate levels in brain in hyperammonemic states are in agreement with this suggestion (9, 18). Though malate dehydrogenase activity in the direction of malate formation is unaffected, it would be of little consequence as malate is not the substrate for citrate synthase and aspartate aminotransferase.

It is quite possible that more than one mechanism may be involved in bringing about the observed changes in the activities of different enzymes in hyperammonemic states. These may be an increase in mitochondrial content (Table III); changes in the phosphorylation-dephosphorylation of enzymes such as pyruvate, isocitrate and α-ketoglutarate dehydrogenases (19, 20) and changes in the membrane fluidity (21). Though an increase in the mitochondrial protein content was observed in the present investigation, further studies are required in this direction.

Changes in the activities of malate dehydrogenase (in the direction of OAA formation) and aspartate aminotransferase in cytosol in hyperammonemic states are similar to those of mitochondria. However, in cytosol malate dehydrogenase is supposed to be involved in the synthesis of malate and it is interesting to note that the enzyme activity in the direction is enhanced in hyperammonemic states. Despite this, production of malate in this compartment would be limited due to the reduction in the amount of aspartate available (c.f. above) and fall in the activity of aspartate aminotransferase in this compartment. Thus, it appears that reduction of malate dehydrogenase activity in mitochondria and of aspartate aminotransferase in mitochondria and cytosol might be the reasons for the failure of malate-aspartate shuttle in hyperammonemic states. Moreover, reduced aspartate aminotransferase activity would affect the production of glutamate which is required for the exchange with mitochondrial aspartate and also for glutamine synthesis. It is interesting to note that addition of either glutamate or aspartate normalized the malate-aspartate shuttle in the primary cultures of astrocytes in the presence of pathophysiological concentrations of ammonium chloride (22). Under in vivo conditions, such a situation is averted by the augmented production of glutamate from the transamination of branched chain amino acid and of oxaloacetate by carbon dioxide fixation (23, 24).

Changes observed in the cytosolic activities of pyruvate dehydrogenase and citric acid cycle enzymes in hyperammonemic states are surprising as this fraction is supposed to be devoid of them. Activities of these enzymes, though less in this fraction when compared to the mitochondria, were enhanced in hyperammonemic state. Such an increase is difficult to explain unless it is assumed that at least some mitochondria have altered

Table VII. Activity Levels and Relative Percentages of Marker Enzymes in Subcellular Fractions of Normal and Hyperammonemic Rats

Enzyme		Cytosol	Mitochondria	Synaptosomes
SDH	N	0.040 ± 0.003	2.8 ± 0.2	0.53 ± 0.04
	SA	0.197 ± 0.015	4.9 ± 0.4	0.73 ± 0.01
		$P<0.001$ +393%	$P<0.005$ +76%	$P<0.001$ +38%
	A	0.330 ± 0.009	5.56 ± 0.71	0.90 ± 0.10
		$P<0.001$ +725%	$P<0.001$ +99%	$P<0.05$ +74%
LDH(1)	N	385 ± 72	79 ± 3	121 ± 9
	SA	295 ± 15	24 ± 3	95 ± 8
		$P<0.001$ −23%	$P<0.001$ −70%	$P<0.005$ −21%
	A	145 ± 29	34 ± 7	108 ± 4
		$P<0.001$ −62%	$P<0.001$ −57%	$P<0.01$ −11%
LDH(2)	N	38 ± 4	23 ± 4	23 ± 3
	SA	69 ± 5	24 ± 2	34 ± 4
		$P<0.001$ +82%	N.S. +4%	$P<0.005$ +48%
	A	48 ± 12	22 ± 3	47 ± 8
		N.S. +26%	N.S. −4%	$P<0.001$ +104%
			Relative Percentages	
SDH	N	1.58 ± 0.26	100 ± 0	19.3 ± 3.5
	SA	4.10 ± 0.76	100 ± 0	15.2 ± 2.6
		$P<0.001$		N.S.
	A	6.00 ± 1.1	100 ± 0	15.7 ± 4.8
		$P<0.001$		N.S.
LDH(1)	N	100 ± 0	21 ± 2.0	31.6 ± 3.7
	SA	100 ± 0	8.2 ± 1.1	32.0 ± 3.4
			$P<0.001$	N.S.
	A	100 ± 0	23.9 ± 4.0	75.6 ± 12.0
			N.S.	$P<0.001$
LDH(2)	N	100 ± 0	63 ± 15	63 ± 14
	SA	100 ± 0	35 ± 6	50 ± 7
			$P<0.005$	N.S.
	A	100 ± 0	47 ± 11	101 ± 23
			N.S.	$P<0.02$

LDH : Lactate dehydrogenase (1) pyruvate → lactate, activity is expressed as μmoles of NADH oxidized/mg protein/hr; (2) lactate → pyruvate, activity is expressed as μmoles of NAD reduced/mg protein/hr. Rest of the legend same as in Table IV.

their buoyant density due to swelling and sediment at higher centrifugal forces or a population of mitochondria degenerate and release their contents in to cytosol in hyperammonemic states. It is interesting to note that such changes have been reported in the mitochondria in hyperammonemic states (25). Despite these changes, availability of substrates and NAD^+ required for these enzymes in cytosol might be inadequate and rate limiting. Hence, such changes may not influence cellular energy metabolism under these conditions.

As synaptosomes used in the present study have both mitochondria and cytosol, changes observed in the activities of pyruvate dehydrogenase and citric acid cycle enzymes might be similar to those described above. A small population of synaptic mitochondria might have also degenerated under these conditions and liberated their contents into synaptoplasm.

One pertinent point to be discussed at this juncture is whether the activities of enzymes measured in vitro especially under optimal conditions serve as representatives of in vivo changes. These enzymes from normal and hyperammonemic rats were measured under identical assay conditions where optimal concentrations of substrates and cofactors and pH are provided. It is apparent that the observed changes in the activities of these enzymes are not experimental artifacts, but have occurred *in situ* and have survived the isolation procedure and hence they may represent changes that have taken place in vivo.

Present study, thus, suggests a derangement in the operation of malate-aspartate shuttle in the hyperammonemic states might be due to the suppression of malate dehydrogenase in mitochondria and of aspartate aminotransferase in mitochondria and cytosol and the

possibility of existence of a small population of mitochondria which are highly vulnerable to ammonia. Further studies are being conducted to localise these changes in the specific cellular compartments of brain.

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