Glutaminase activity in various tissues and their relative organ weights in long term (90 days) Alloxan Diabetic Rats

V Vijay, RT Kashinath[1], HP Sandhya[2], V Indumati, MS Shekanawar, M Amareshwara, Rajeshwari, D Krishnaswamy

Department of Biochemistry VIMS, Bellarly, [1]Department of Biochemistry SIMS and RC, Shimoga, [2]Department of Physiology VIMS, Bellary, Karnataka, India

Abstract

Introduction: Glutaminase catalyses the glutamine, to glutamic acid and ammonia. This catabolism by glutaminase mainly occurs in liver, kidneys, brain and small intestines. Diabetes is a chronic metabolic disorder and there are several studies related to glutaminase activity in various tissues of acute/ short term diabetic rats. Little is known about the glutaminase activity in long term diabetic rats.

Methods: Glutaminase activity in liver, kidney, brain and small intestines of six normal and six diabetic rats were determined. Glutamine was incubated in the tissue homogenates (containing glutaminase) and ammonia liberated was determined by Berthelot's reaction and its activity was expressed as µg NH_3 released/g tissue/hour.

Results: Glutaminase activity in the present study was significantly increased ($p<0.02$) in liver and small intestine whereas insignificant increase was seen in kidneys and brain homogenates of diabetic rats. The body weight of rats was significantly decreased ($p<0.0001$) along with significant increase in ratios of various organs to bodyweight (relative organ weights).

Conclusion: It is evident from the study that glutaminase activity is increased in diabetic liver and intestines in long-term diabetic rats, similar to short term diabetes, may be to metabolize and channelize the excess glutamine for gluconeogenesis. Further research should be carried out to study the causes and effects of inactivation of glutaminase enzyme in the diabetic tissues and its relation to diabetic complications like diabetic nephropathy and encephalopathy.

Keywords: brain glutaminase, liver glutaminase, kidney glutaminase, Intestinal glutaminase, gluconeogenesis.

Introduction

The major problem associated with diabetes mellitus is continued production of glucose by gluconeogenesis, even in the presence of hyperglycemia [1]. Glutamine, being a glucogenic amino acid, can be converted to glucose in liver and kidneys. The first step of this conversion is formation of ammonia and glutamic acid from glutamine, catalysed by a mitochondrial enzyme, glutaminase (L-Glutamine aminohydrolase; E.C 3.5.1.2). This catabolism by glutaminase primarily occurs in liver, kidneys, brain and small intestines [2]. There are two isoenzymes of glutaminase; Liver-type glutaminase is found in periportal hepatocytes where it couples ammonia production with urea synthesis and Kidney–type glutaminase which is found in kidneys, brain, intestines, lymphocytes etc., where the ammonia is released without further metabolism. Liver-type glutaminase is increased in starvation, diabetes, high protein diet and kidney type glutaminase is increased in response to metabolic acidosis [3].

One of the most important roles of glutaminase is found in the axonal terminals of neurons in the central nervous system (CNS). Glutamate is the most abundantly used excitatory neurotransmitter in the CNS. After being released into the synapse for neurotransmission, glutamate is rapidly taken up by nearby astrocytes, which convert it to glutamine. This glutamine is then supplied to the presynaptic terminals of the neurons, where glutaminases convert it back to glutamate for loading into synaptic vesicles. Glutaminase is the main glutamate-producer enzyme in brain [6] and is essential for glutamatergic and

Address for Correspondences

Dr. Vijay V. Associate Professor of Biochemistry, Vijayanagara Institute of Medical Sciences, Bellary -583104, Karnataka, India
E-mail:-ursdrvijay@yahoo.com

GABAergic transmissions. It is also essential in biosynthesis of energy, proteins, glutathione and in production of basic metabolites such as GABA in brain. Glutamate (an excitotoxic substance in brain) and ammonia, the products of glutamine catabolism by glutaminase, are hazardous compounds when their generation is beyond normal physiological thresholds. So their generation in brain is carefully regulated.

Under normal physiological conditions, the major site of glutamine utilization is small intestine, where it is used as respiratory fuel by absorptive columnar epithelial cells [5-9]. Glutaminase has a central role in intestinal metabolism: the products of the reaction catalyzed by glutaminase can be transaminated, catabolized to yield energy or used for the biosynthesis of pyrimidine nucleotides [10]. In normal rats, the highest glutaminase activity along the length of gastrointestinal tract has been measured in the small intestine [11]. Lower but still substantial activity (15% of total glutaminase activity) has been found in large intestine. Glutaminase activity distribution along the gastrointestinal tract in humans is similar to that in rats [12]. In insulin deficiency, glutamine released from muscles is used as precursor for gluconeogenesis in small intestine [13]. Several researchers have studied glutaminase activity in liver, kidney, brain and intestines in short term (7 to 10 days) diabetic rats. Hence the present study was undertaken to study the effect of long-term (90 days) hyperglycemia on activity of glutaminase in liver, kidneys, brain and small intestines in alloxan diabetic rats.

Materials and methods

Chemicals:

Alloxan and L-glutamine were procured from Sigma-Aldrich Chemical Company (USA). All other chemicals employed were of analytical grade.

Animals:

Adult albino rats of both sexes, weighing 300-350g, were randomly selected and used for the present investigation. The animals were maintained on a standard rat feed supplied from Pranav agro industries, Pune, India. Water was provided ad libitum to the rats.

Ethical clearance:

The experiments were conducted according to the norms approved by Ministry of Social Justice and empowerment, Government of India, and Institutional Animal Ethics Committee (IAEC) guidelines.

Induction of Diabetes:

Diabetes was induced in the overnight fasted rats, by a single intraperitoneal injection of freshly prepared alloxan (150mg/kg body wt.)[14], in sterile normal saline. The animals were considered diabetic if their blood glucose levels were above 250mg/dl and urine showed glucosuria consistently.

Grouping of animals:

The rats were divided into two groups comprising six rats in each group, as follows:

Normal control rats – were fed on normal rat feed and water ad libitum, daily for 90 days.

Diabetic (hyperglycemic) rats – were fed on normal rat feed and water *ad libitum*, daily for 90 days. These rats received a very low dose of insulin (2 units of Human Mixtard insulin subcutaneously, with aseptic precautions, daily for survival purpose). Rats, whose blood glucose was consistently more than 300mg/dl were included in the study.

Collection of blood, tissues and their processing:

On completion of the stipulated period, the rats of both the groups were anaesthetized using anesthetic ether. The rats were immediately sacrificed by cervical dislocation and blood was collected in heparinised tubes from internal jugular vein. Brain was dissected out, and blood stains were carefully washed with ice cold normal saline. Kidneys and liver were dissected and blood stains were removed with smooth blotting. Small intestine (3 cms of jejunum) was dissected, trimmed of connective tissue, thoroughly washed in ice cold normal saline, through the lumen, to flush out its contents and residual saline was removed by gentle squeezing. These dissected tissues were preserved in small beakers tightly closed with aluminium foils at 0 degree centigrade, till further use. A part whole blood was employed for estimation of carbohydrate content in hemoglobin (glycated-Hb)[15,16] and the remaining blood was centrifuged for 6 minutes at 3000 rpm. The clear plasma was transferred to a clean tube and was employed for estimation of plasma glucose [17].

One part of kidney, liver, intestine (thoroughly

washed and cut into small pieces) and one half hemisphere of whole brain were homogenised, using Potter Elvehjam homogenizer, with 9 parts of ice cold Phosphate buffer (pH 7.4) for 10 minutes. The homogenates were centrifuged for 5 minutes at 3000rpm and clear supernatant was employed for determination of Glutaminase activity (Table 1) .

Estimation of glutaminase activity in the tissue homogenates (18):

Glutamine was incubated with the tissue homogenates containing glutaminase, which catalyses it into glutamic acid and ammonia. Ammonia released was determined by Bethelot's reaction [18] and glutaminase activity was expressed as microgram of ammonia released per gram tissue per hour (µg NH_3 /g tissue/hour). The results were expressed as mean ± standard deviation. Statistical analysis was done by using students 't' test .

Table1. showing Body weight of rats and relative organs' weight (ratios of various organ weights to body weight), of normal control and diabetic rats:

	Normal Control rats (n=6)	Diabetic rats (90 days) (n=6)
Body weight (gms)	323.81 ± 55.65	217.85**** ± 31.40
Liver / body weight ratio (liver all lobes)	0.03 ± 0.006	0.042** ± 0.003
Kidney / body weight ratio (both kidneys)	0.0071 ± 0.0009	0.0091*** ± 0.0008
Brain / body weight ratio (whole brain)	0.0062 ± 0.004	0.0067* ± 0.005
Intestine/ body weight ratio (3 cms of jejunum)	0.0089 ± 0.001	0.0111*** ± 0.005

Note: 1. Number in parenthesis indicate the number of animals in each group.
2. The values are expressed as their mean ± SD
3. Significance level : *p<0.02; ** p < 0.05; *** p < 0.001; ****p<0.0001

Table 2. showing Plasma glucose , Glycated hemoglobin and Glutaminase activity in various tissues of Group I and Group II rats

	Normal Control rats (n=6)	Diabetic rats (90 days) (n=6)
Plasma Glucose (mg/dl)	114.89 ± 19.66	493.6*** ± 87.81
Glycated hemoglobin (%)	3.9 ± 1.2	16.2*** ± 1.5
Hepatic Glutaminase (µg NH_4/g tissue/hr)	9.70 ± 3.19	12.48** ± 4.28
Renal Glutaminase (µg NH_4/g tissue/hr)	33.33 ± 5.89	38.62 ± 7.43
Brain Glutaminase (µg NH_4/g tissue/hr)	49.92 ± 6.79	58.24 ± 13.58
Intestinal Glutaminase (µg NH_4/g tissue/hr)	35.13 ± 17.01	47.14** ± 15.36

Note: 1. Number in parenthesis indicate the number of animals in each group.
2. The values are expressed as their mean ± SD
3. Significance level : *p<0.02; ** p < 0.05; *** p < 0.001; ****p<0.0001

Results

From the table 1, it is evident that the diabetic rats show significant decrease ($p<0.0001$) in body weight and significant increase in the relative organ weights (ratios between weights of kidneys, brain, intestine, liver and body weight) when compared to normal control rats. From the table 2, it is evident that the diabetic rats show significant increase ($p<0.001$) in plasma glucose and glycated hemoglobin levels as well as significant increase ($p<0.02$) in hepatic and intestinal glutaminase levels and insignificant increase in renal and brain glutaminase levels, when compared to normal control rats.

Discussion

In the present study, administration of alloxan (150mg/kg body weight) induced hyperglycemia in the albino rats as evidenced by elevated plasma glucose levels and glycated hemoglobin levels in group II rats (refer table 2). The levels of glycated hemoglobin have been shown to be an important parameter of chronic glycemic control in diabetes. The decrease in body weight of diabetic rats is due to increase in the protein catabolism mainly in skeletal muscles that helps to channel amino acids for gluconeogenesis, decrease in protein uptake and increased lipolysis due to insulin deficiency [19]. Increase in the relative weight of organs (ratios between weights of kidneys, brain, intestine, liver and that of body weight) in diabetic rats (refer table 1) is due to increase in lipogenesis and lipid deposition in organs like liver and kidneys [20], over expression of growth factors leading to hypertrophy, inflammatory process, edema, etc in chronic diabetic state [21].

In diabetes mellitus, there is continued production of glucose by gluconeogenesis, even in the presence of hyperglycemia [1]. Hence in diabetes, there is increased protein catabolism mainly in skeletal muscles that causes increase in alanine and glutamine levels in blood. Alanine is readily converted to glucose in liver by gluconeogenesis, but glutamine is converted to glutamate by glutaminase, partly in GIT (small intestines) and then it is converted to glucose [13]. Hence it is expected that there is increase in liver and intestinal glutaminase activity in diabetic state. In the present study we found a significant increase ($p<0.02$) in hepatic glutaminase activity, which is in agreement with the studies of Stephen AS **(1)** and Buniatian GK [22] and significant increase ($p<0.02$) in small intestinal glutaminase activity, which is in agreement with the studies of Ardwani MS[23]. Hence increase in hepatic and intestinal glutaminase may be due to increase the catabolism of glutamine to glutamate, which is an essential amino acid for gluconeogenesis in diabetic state.

Our study showed insignificant change in glutaminase activity in brain homogenate of diabetic rats, which are in agreement with the studies of Buniatian [22] and V Lellos [6].

Glutaminase is also expressed in the epithelial cells of the renal tubules, where the produced ammonia is excreted as ammonium ions. This excretion of ammonium ions is an important mechanism of renal acid-base regulation. In uncontrolled diabetes, there is acidosis due to increased ketogenesis and also there is increase gluconeogenesis in kidneys, both of which can cause increase in renal glutaminase activity. Our study showed an insignificant increase in renal glutaminase activity which is in contrast to the studies of Curthoys NP [3,24]. This insignificant change in renal glutaminase activity in diabetic rats may be because of:

1) Difference in estimation of glutaminase, as the other studies have determined phosphate dependent/phosphate-activated glutaminase (PAG) or mitochondrial glutaminase in kidneys and brain [6,22,25-27], whereas in our study we estimated glutaminase activity in tissue homogenates without using activators like phosphates etc, which can be taken as limitation of the present study, and 2) Duration of diabetes; in our study it was in 90 days diabetic rats and other studies showed significant increase in short term/acute (less than 10 days) diabetic rats [6, 25].

Conclusion

It is evident from our study that the glutaminase activity is significantly increased ($p<0.02$) in liver and intestines in long term (90 days) diabetic rats, similar to short term/ acute diabetic rats, which may be to metabolize and channelize the excess glutamine from protein catabolism, for gluconeogenesis. Further research should be carried out to study the causes and effects of inactivation of glutaminase enzyme in the tissues like kidney and

brain of chronic diabetic rats and their relation to diabetic complications like diabetic nephropathy and encephalopathy.

References

1. Stephen A, Squires H, Stephen E, Colin M, Margaret EB, John TB. Regulation of Hepatic Glutaminase in the Streptozotocin-Induced Diabetic Rat. Diabetes.1997; vol.(46):1945-1949.
2. Wayne GH, Richard AS, Norman PC. Comparison of the phosphate- dependent glutaminase obtained from rat brain and kidney. Biochem. J. 1985; 229: 399-408.
3. Curthoys NP, Watford M. Regulation of glutaminase activity and glutamine metabolism. Annu Rev Nutr. 1995;15:133-59.
4. Alba-Loureiro TC, Hirabara SM, Mendonca JR, Curi R, Pithon-Curi TC. Diabetes causes marked changes in function and metabolism of rat neutrophils. J Endocrinol. 2006; 188: 295-303.
5. Malcolm W, Emily J. E and Eleanor M. S. The regulation of glutamine and ketone-body metabolism in the small intestine of the long-term (40-day) streptozotocin-diabetic rat. Biochem. J. 1987; 242:61-68.
6. Lellos V, Moraitou M, Tselentis V, Philippidis H, Palaiologos G. Effect of Starvation or Streptozotocin-Diabetes on Phosphate-Activated Glutaminase of Different Rat Brain Regions. Neurochemical Research. 1992;17(2):141-145.
7. Márquez J. Brain glutaminases. BioMolecular Concepts. 2010;1(1):3–15.
8. Márquez J, Tosina M, Rosa V, Segura JA, Alonso FJ, Matés JM. Campos-Sandoval JA. New insights into brain glutaminases: beyond their role on glutamatergic transmission. Neurochem Int. 2009;55(1-3):64-70.
9. Olalla L, Gutiérrez A, Campos JA, Khan ZU, Alonso FJ, Segura JA, Márquez J, Aledo JC (Aug 2002). "Nuclear localization of L-type glutaminase in mammalian brain". *J. Biol. Chem.*2002; 277 (41): 38939–38944.
10. McCauley R, Kong SE, Heel K, Hall JC. The role of glutaminase in the small intestine. Int J Biochem Cell Biol. 1999; 31(3-4):405-13.
11. James LA, Lunn PG, Elia M. Glutamine metabolism in the gastrointestinal tract of the rat assess by the relative activities of glutaminase (EC 3.5.1.2) and glutamine synthetase (EC 6.3.1.2). Br J Nutr 1998; 79: 365-372.
12. James LA, Lunn PG, Middleton S, Elia M. Distribution of glutaminase and glutamine synthetase activities in the human gastrointestinal tract. Clin Sci (Lond) 1998; 94: 313-319.
13. Rajas F, Croset M, Zitoun C. "Induction of PEPCK gene expression in insulinopenia in rat small intestine". Diabetes. 2000;49:1165-1168.
14. Mohammed FA. Antidiabetic Activity of Vinca rosea Extracts in Alloxan-Induced Diabetic Rats". International Journal of Endocrinology.2010;(2010):1-6.
15. Dubois M.Colorimetric methods for determination of sugars and related substances. Anal. Chem.1956;28:350-356.
16. Vijay V, Vickram, Kashinath RT. Effect of Diallyl disulphide protein and lipid glycation in alloxan diabetic rat kidney. Journal of Advance Researches in Biological Sciences,2010;2(2):15-18.
17. Varley H, Gowenloch AH Bell M. Glucose estimation Practical Clinical Biochemistry. 5th London: Heimann Professional publishing Ltd.1991.
18. Varley H, Gowenloch A H and Bell M. eds.. NPN substances Practical Clinical Biochemistry. 5th London: Heimann Professional publishing Ltd. 1991.
19. Textbook of Biochemistry by D. M. Vasudevan, Srikumari S, 6nd ed, Chapter 24, Regulation of blood glucose, Insulin and Diabetes mellitus, Jaypee Brothers, Medical (P) Ltd., New Delhi,2011; pg 274-291.

20.Yadav UCS, Moorthy K Baquer NZ. Effects of sodium-orthovanadate and Trigonella foenum-graecum seeds on hepatic and renal lipogenic enzymes and lipid profile during alloxan diabetes; J. Biosci.2004;(29):81–91.

21.Kota SK, Meher LK, Jammula S, Kota SK, Krishna S, Modi KD. Aberrant angiogenesis: The gateway to diabetic complications. Indian J Endocr Metab 2012;16:918-30.

22.Buniatian GK, Egian VB, Turshian GA, Akopian GE, Safrazian SS.Glutamine synthetase and glutaminase activity in the brain and liver homogenates and subcellular fractions of rats with alloxan diabetes. Vopr Biokhim Mozga. 1974;9:17-24.

23.Ardawi MS. The maximal activity of phosphate-dependent glutaminase and glutamine metabolism in the colon and the small intestine of streptozotocin-diabetic rats. 1987;30(2):109-14.

24. Lemieux G, Aranda MR, Fournel P, Lemieux C. Renal enzymes during experimental diabetes mellitus in the rat. Role of insulin, carbohydrate metabolism, and ketoacidosis.Can J Physiol Pharmacol. 1984;62(1):70-5.

25. Malcolm W, Eleanor MS, Emily JE. The regulation of phosphate-activated glutaminase activity and glutamine metabolism in the streptozotocin-diabetic rat. Biochem. J. 1984;224:207-214.

26. Norman PC. Phosphate-Dependent Glutaminase from Rat Kidney Cause of Increased Activity in Response to Acidosis and Identity with Glutaminase from Other Tissues . Archives of biochemistry and biophysics.1976;172:162-167.

27.Suzuki S. "Phosphate-activated glutaminase (GLS2), a p53-inducible regulator of glutamine metabolism and reactive oxygen species". *Proc. Natl. Acad. Sci. U.S.A.2010;* 107(16):7461–6.

Source of Support : **Nil**
Conflict of Interest : **None Declared**