Hyperglycemia and Starvation: Contrast in Rat Brain Gluconeogenic Amino Acids and Glycogen[1] (36820)

RAUL A. WAPNIR, MICHAEL S. GORBACK, AND EILVER H. EDWARDS

Rosewood State Hospital, Owings Mills, Maryland 21117; and University of Maryland School of Medicine, Department of Pediatrics, Baltimore, Maryland 21201

Streptozotocin, when administered intravenously in doses of 50 to 100 mg/kg has been reported to induce diabetes mellitus in rats and other animal species by producing degranulation and necrosis of pancreatic beta cells (1, 2). The cellular damage resulted in consistent hyperglycemia, glucosuria, mild ketonuria and sharp reduction of pancreatic immunoreactive insulin.

It has been known since 1950 that the concentration of brain free amino acids can be affected by the circulating levels of glucose. This was particularly striking in the case of insulin-induced hypoglycemia (3–5). Also, it has been shown recently that 24 to 48 hr of starvation decreased free alanine in the brain of both newborn and suckling rats. The same effect was produced on glycine and glutamate, but only in the newborn. In contrast, starvation for up to 120 hr in the adult rat was associated with an increase in free amino acids in brain. However, glycogen concentrations were not affected by fasting in any age group (6).

In the studies reported here, we have correlated streptozotocin-induced hyperglycemia and hyperketonemia with the levels of three gluconeogenic amino acids, either free in brain or in blood as an approach to understand the utilization of ancillary sources of energy by the brain during a stress affecting energy metabolism.

Materials and Methods. Adult male Wistar rats weighing 250 to 300 g were intravenously injected with 65 mg/kg of streptozotocin.[2] The drug was dissolved just before use in 0.5 ml of sterile 0.05 *M* sodium citrate, pH 4.5. The animals were offered a commercial feed[3] and water *ad libitum*. One week later the rats were anesthesized with 60 mg/kg of Nembutal, according to the technique of Prasannan, Rajan and Subrahmanyam (7) and decapitated. The brain was immediately excised and one hemisphere was used for glycogen assay (7) and the other was homogenized with 4 vol of 95% ethanol for amino acid determination (8) or extracted for DNA assay (9). Heparinized blood was collected for glucose (10), total ketones (11) and amino acid analyses (8). Other rats of the same source and weight were fasted for 72 hr, although water was freely available. The animals were sacrificed and tissues were processed and analyzed as indicated above.

Results. Streptozotocin-treated rats exhibited a consistent hyperglycemia and hyperketonemia ($p < .001$, Table I). In animals starved for 72 hr, blood glucose values were significantly lower and ketones higher than those in the fed controls. Nevertheless, free amino acids in the circulation were essentially the same in diabetic, fasted or fed animals, except for alanine in starved rats ($p < .05$, Table I).

Brain free amino acids and glycogen changes occurring in starved and diabetic rats are shown in Table II. While fasted rats exhibited a significant rise in glycine and glutamate, streptozotocin-treated animals manifested changes only in glutamate, as compared with controls. However, while starvation did not affect brain glycogen concentration, glycogen did rise very significantly

[1] Supported in part by NIH Grants HD 03959-04 and HD 06291-01 and the John A. Hartford Foundation.

[2] Gift from Dr. W. E. Dulin, The Upjohn Co., Kalamazoo, MI, Lot #9164-VDV-131.

[3] Purina Lab Chow, Ralston Purina Co., St. Louis, MO.

TABLE I. Blood Glucose, Ketones and Free Amino Acids in Fed, Starved and Diabetic Rats.

Treatment group[a]	Glucose (mg/100 ml)	Ketones (mg/100 ml)	Alanine (μmoles/ml)	Glycine (μmoles/ml)	Glutamate[b] (μmoles/ml)
Fed controls (14)	122 ± 9	2.6 ± 0.4	0.44 ± 0.02	0.24 ± 0.03	1.08 ± 0.06
Starved (10)	92 ± 4[c]	8.4 ± 1.7[c]	0.57 ± 0.04[c]	0.35 ± 0.05	1.20 ± 0.12
Diabetic (9)	399 ± 21[d]	68.5 ± 36.3[d]	0.58 ± 0.09	0.24 ± 0.03	0.92 ± 0.04

[a] All values are means ± SEM. The number of animals is given in parentheses.
[b] The values for glutamate include both glutamate and glutamine.
[c] $p < .05$; [d] $p < .001$ compared to fed controls.

($p < .01$, Table II) in diabetic rats, to a level double that of control animals.

Discussion. Hyperglycemia and the concomitant hyperketonemia had no effect on the three circulating gluconeogenic amino acids monitored in these experiments. Simultaneously, brain glutamate increased over base line values, in parallel, but not to the same extent as the increase in blood glucose and ketones. This suggests that the brain pool of this amino acid reflected the changes in glucose and ketone concentrations rather than the circulating levels of glutamate/glutamine, which apparently were not affected by the experimental diabetes. More strikingly, there was a significant increase in glycogen stores. Only in very specific cases brain glycogen is known to be altered. Depletion has been reported to occur during insulin-induced hypoglycemia (12), extreme starvation of newborn pups (6, 13) or brain perfusion by glucose-free solutions (14). Conversely, increases in glycogen levels have been reported only rarely, *e.g.*, in electroshock (15) or with hydrocortisone treatment (12).

Changes in brain free gluconeogenic amino acids take place not only as a consequence of a decline in the circulating levels, but also when the muscle protein is unable to provide a continuous inflow of alanine to the liver through the "alanine cycle" for gluconeogenesis (16). This is observed in the starved newborn rat. In the immature animals, it would appear that endogenous brain alanine can be promptly utilized as an ancillary source of energy. This does not occur with glycine and glutamate which only decline in terminal situations (6).

Data presented here suggest that higher circulating levels of glucose and ketones may stimulate glycogen synthesis and induce the accumulation of gluconeogenic intermediates. In contrast, mild starvation producing a threefold increase in blood ketones and a decline of blood glucose of 20–25 mg/100 ml had no influence on brain glycogen or depleted brain glutamate stores. In fact, brain glutamate appeared to be either affected by the influx of ketones or by a partial block in the tricarboxylic acid cycle and be a sensitive indicator of changes in the supply of glucose to the brain. These experiments suggest that hyperglycemia may be of critical importance in affecting brain metabolites. The possible

TABLE II. Brain Free Amino Acids and Glycogen in Fed, Starved and Diabetic Rats.

Treatment group[a]	Alanine (μmoles/mg DNA)	Glycine (μmoles/mg DNA)	Glutamate[b] (μmoles/mg DNA)	Glycogen (mg/mg DNA)
Fed controls (8)	0.23 ± 0.02	0.28 ± 0.01	4.46 ± 0.29	0.09 ± 0.01
Starved (14)	0.25 ± 0.01	0.41 ± 0.04[c]	6.71 ± 0.59[c]	0.07 ± 0.01
Diabetic (9)	0.27 ± 0.01	0.28 ± 0.03	5.43 ± 0.23[c]	0.18 ± 0.02[c]

[a] All values are means ± SEM. The number of animals is given in parentheses.
[b] The values for glutamate include both glutamine and glutamate.
[c] $p < .01$ compared to fed controls.

influence of glycogen and glutamate accumulation on brain metabolism and function in pathological conditions remains to be elucidated.

Summary. Adult rats made diabetic with streptozotocin had an increased concentration of brain free glutamate and glycogen but showed no changes in brain free alanine and glycine. These alterations paralleled higher circulating levels of glucose and ketones. Conversely, starved rats accumulated glycine and glutamate in the brain, although glycogen stores were not affected.

1. Junod, A., Lambert, A. E., Orci, L., Pictet, R., Gonet, A. E., and Renold, A. E., Proc. Soc. Exp. Biol. Med. **126**, 201 (1967).

2. Junod, A., Lambert, A. E., Stauffacher, W., and Renold, A. E., J. Clin. Invest. **48**, 2129 (1969).

3. Dawson, R. M. C., Biochem. J. **47**, 386 (1950).

4. Cravioto, R. D., Masien, G., and Izquierdo, J. J., Proc. Soc. Exp. Biol. Med. **78**, 856 (1951).

5. DeRopp, R. S., and Snedeker, E. H., J. Neurochem. **7**, 128 (1961).

6. Wapnir, R. A., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **31**, 680, Abs. (1972).

7. Prasannan, K. G., Rajan, R., and Subrahmanyam, K., Indian J. Med. Res. **51**, 703 (1963).

8. Blackburn, S., "Amino Acid Determination. Methods and Techniques," p. 219. Dekker, New York (1968).

9. Schneider, W. C., *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. 3, p. 680. Academic Press, New York (1957).

10. Marks, V., Clin. Chim. Acta **4**, 395 (1959).

11. Bessman, S. P., and Anderson, M., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **16**, 154 (1957).

12. Timiras, O. S., Woodbury, D. M., and Baker, D. H., Arch. Int. Pharmacodyn. Ther. **105**, 450 (1956).

13. Wapnir, R. A., Tildon, J. T., and Cornblath, M., Pediat. Res. **6**, 395 (1972).

14. Mukherji, B., Turinsky, J., and Sloviter, H. A., J. Neurochem. **18**, 1783 (1971).

15. Chance, M. R. A., and Yaxley, D. C., J. Exp. Biol. **27**, 311 (1950).

16. Felig, P., Pozefsky, T., Marliss, E., and Cahill, J. F., Jr., Science **167**, 1003 (1970).

Received June 16, 1972. P.S.E.B.M., 1972, Vol. 141.