Pakistan J. Sci. Ind. Res, Vol. 31, No. 5, May 1988

EFFECT OF GLUCOSE ON THE DISTRIBUTION OF TRYPTOPHAN IN SERUM LIVER AND BRAIN

Abdur Rehman Siddiqi, Darakhshan J. Haleem and M.A. Haleem

Department of Biochemistry, Biophysics Unit, University of Karachi, Karachi - 32

(Received November 27, 1986; revised **June** 8, 1988)

Effect of glucose administration has been studied on the distribution of tryptophan in serum, liver and brain of rabbits. Glucose administration caused a considerable fall in serum free tryptophan with a concomittant increase in tryptophan binding to serum albumin. A significant increase of total tryptophan was found in serum, liver and brain.

Key words : Effect of glucose, Tryptophan.

INTRODUCTION

Tryptophan is one of the most important of the amino acids which have to be supplied to the brain from the blood [1]. The concentration of serotonin a putative neurotransmitter in mammals. CNS varies as a result of physiological changes in the availability of its precusor tryptophan[2]. Since tryptophan is a long neutral amino-acid (LNAA), thus shares a competitive transport carrier with such others LNAAs as tyrosine, phenylalanine, leucine, isoleucine and valine [3-7]. However, workers have correlated the brain tryptophan uptake to its ratio to other LNAAs, until recently there was some disagreement between different research groups as to which of (a) plasma tryptophan (b) plasma total tryptophan (c) the plasma tryptophan/LNAAs ratio or (d) the plasma free tryptophan/LNAAs ratio might be the best predictor of tryptophan entering the brain [8].

We studied the effect of glucose mainly on serum and brain tryptophan levels to correlate it with the pharmacological behaviour of drug. Australian rabbits were selected for this investigation.

MATERIALS AND METHODS

Young Australian rabbits 1 to 1.5 kg. were housed 3 per cage. They had free access to food (grass) and water. A group of five animals were injected intraperitoneally with glucose (dose, 200 mg/kg of body weight). Rabbits were sacrified by cervical dislocation, blood was collected, centrifuged after 10 minutes and serum was frozen and subsequently assayed for tryptophan. Brain and liver were quickly removed and frozen at O^{O} until assayed for tryptophan.

Estimation of total tryptophan. Total tryptophan was estimated in serum, liver and brain as described by Denkla & Deway [9], except that the addition of the $FeCl_3$ solution was delayed until just prior to boiling as recommended by Bloxam & Warren [10]. Estimations were made in whole serum and 10% homogenates of liver and brain in T.C.A. (12%). 2 ml of 10% T.C.A., was added to each sample, after mixing and centrifugation to precipitate the protein, the supernatant was transferred to screw caped boiling tube. 0.2 ml of 2% formaldehyde was added to each tube just prior to incubation for 1 hour at 100^{O} C in water bath, content were added to 0.1 ml $6x10^{-3}$M $FeCl_3$ (prepared in 10% T.C.A.). After incubation for 1 hour the tubes were cooled rapidly at room temperature. The fluorescence was measured at excitation 365.5 nm and emission at 480 nm, using Jasco-FP 500 spectrofluoremeter.

Estimation of free tryptophan. A. 0.4 ml volume of freshly separated serum was placed in dialysis tube and ultrafiltration membrane (Union Carbide Corporation, Chicago) were mounted on tube. The tube was inverted in small sample tube containing 1 ml of 0.1 M phosphate buffer, and incubated at 4^{O}C for 22 hours. 0.1 ml of dialysate was used for the estimation, using same procedure as described for total tryptophan.

RESULT

The effect of intraperitoneal administration of glucose (200 mg/kg) on tryptophan binding and concentration of total tryptophan in serum, liver and brain are shown in Table 1. Results show that the concentration of free tryptophan decreased to 25%, whereas total tryptophan in serum, liver and brain increased to 83.3%, 35.1% and 31.1% respectively.

Table 1. Effect of glucose on tryptophan protein binding in serum, and on the concentration of tryptophan in serum, liver and brain. Results are expressed as mean-S.E of 8 animals. Glucose (200 mg/kg) was injected intraperi-

toneally. Control animals received an equal volume of saline. The analysis was performed in Australian rabbits.

Table 1

Treatment	Serum (μg/ml)		Liver (μg/gm)	Brain (μg/gm)
	Total	Free	Total	Total
Control animal	10.27±0.50	1.08±0.05	8.47±0.31	2.44±0.10
Glucose injected	18.82±0.81	0.81±0.03	11.44±0.36	3.20±0.12
% Change	83.3% increased	25% decreased	35.1% increased	31.1% increased

DISCUSSION

The intraperitoneal administration of glucose leads to an increase 83.3% ($p < 0.05$) in total serum tryptophan level (Table I showing agreement with previous findings [11-13]).

Results (Table 1) shows that the serum free tryptophan concentration decreased to 25%. Our data confirmed the reports [14-15] that the administration of carbohydrate or glucose cause only relatively small decrease in serum free tryptophan (albumin unbound tryptophan [16]).

It is suggested that the administration of glucose leads to elicit insulin secretion from pancreas as described by other workers, thus when insulin secreted after glucose administration, lowers plasma non-esterified fatty acid levels (largely by stripping the non esterified fatty acid of albumin), it increases the ability of the albumin to bind more tryptophan. Hence the plasma concentration of albumin bound tryptophan rises, partly or totaly compensating for all the fall in the smaller free tryptophan pool. The tryptophan bound to albumin is almost as accessible to the brain free tryptophan [17]. Probably because the affinity of the blood brain barrier long neutral amino acid transport system for tryptophan [18]. Hence carbohydrate consumption, which increased serum total tryptophan while decreasing the concentration of other long neutral amino acid, is able to elevate brain tryptophan 31.1% ($p < 0.05$, Table 1,) and to enhance the synthesis and release of serotonin [19-20].

Normally insulin that regulates the carbohydrate metabolism on one hand, promotes the uptake of amino acids in various tissues (including liver, muscles) on the other hand. Previous studies indicate that even lower doses of glucose can elicit the secretion of sufficient insulin to activate the receptor that affect the flux of long neutral amino acids between the plasma and such tissues as skeletal muscle [21]. The insulin dependent uptake of the branched amino acids into muscle and their subsequent transamination or incorporation into proteins constitute the major mechanism for retarding the increase in their plasma level that would otherwise occur after protein ingestion, in as much as these compounds are metabolized only marginally in the liver [22-23]. In contrast dietary phenyl alanine, tyrosine and methionine are removed from the blood stream both by insulin mediated tissue uptake and hepatic metabolism. This difference probably explains, the greater amplitude of the dietary rhythms in plasma branched chain amino acids and their greater percent decrease after glucose consumption.

It thus seems that carbohydrate has two actions on the availability of tryptophan to the tissue; first there is well established insulin mediated increase in the transport of the free amino acids into some tissues notably muscle; second the reduction in non-esterified fatty acids following glucose administration, reduces the availability of free tryptophan by increasing its binding to albumin. Our work supports the notion that considerable increase of insulin concentration is needed to alter tissue uptake of aromatic amino acids, in order to double the rate of entry of tryptophan into rabbit's brain. Thus the ingestion or administration of carbohydrate via insulin secretion increased the binding affinity of albumin to bind with tryptophan, is confirmed. This enhancement in tryptophan binding caused a decrease in free serum tryptophan concentration (25%). Despite the serum free tryptophan fall; the ratio of total tryptophan concentration to other long neutral amino acids increased that resulted in an increased brain tryptophan concentration.

REFERENCES

1. G. Curzon and P.J. Knott, Crit, Rev. Toxicol., **5**, 145 (1977).
2. J.D. Fernstrom and R.J. Wurtman, Science, **173**, 149 (1971).
3. R. Blasberg and A. Lajtha, Arch. Biochem. Biophys., **112**, 361 (1965).
4. M. Daniel and O.E. Pratt, J. Physiol., **284**, 42 (1978b).
5. M. Daniel and O.E. Pratt, J. Physiol., **282**, 9 (1978a).
6. O.E. Pratt, J. Neural. Trans. (Supplement), **15**, 29 (1979a).
7. O.E. Pratt, *In, Advance, Perinatal, Neural, Korobkin,* R. and Guilleminautte (Spectrum Publication, New

York, 1979b), Ist ed., pp. 21-55.
8. Peter D. Leathwood, Bibthia. Nutr. No. **38,** 54-71 (1986)
9. W.D. Denkla amd H.K. Dewey, J. Lab. Clin. Med., **69,** 621 (1974).
10. D.L. Bloxam and W.H. Warren, Analy. Biochem., **60,** 621 (1974).
11. J.D. Fernstrom and R.J. Wurtman, Science, **174,** 1023 (1971).
12. J.D. Fernstrom and R.J. Wurtman, Science, **178,** 414 (1972).
13. M. Daniel, E.R. Love, S.R. Moorhouse and D.E. Pratt, J. Physiol., **312,** 551 (1981).
14. D. Lipsett, B.K. Madras and R.J. Wurtman, Life Science, **12,** 57 (1973).
15. R.D. Martin, C. Maurren, B. Glaeser and R.J. Wurtman, Metabolism, **31,** 937 (1982).
16. R.H. McMenamy and J.L. Oncley, J. Biol. Chem., **223,** 1436 (1958).
17. A. Yuwiler, W.H. Oldendrof and E.J. Geller, Neurochem., **28,** 1015 (1977).
18. W.M. Pardridge and W.H. Oldendrof, J. Neural. Trans., **15,** (Suppl.) 43 (1979).
19. A. Carlsson and M. Lindquist, J. Neural. Trans., **33,** 23 (1972).
20. J.D. Fernstrom and R.J. Wurtman, Science, **173,** 149 (1971).
21. T. Posefsky, P. Felig and J.D. Tobin, J. Clin. Invest., **48,** 2273 (1969).
22. L.L. Miller, Amsterdam, Elsevier, pp. 708-728 (1962)
23. J. Wahren, P. Felig and L. Hagenfeldt, J. Clin. Invest. **57,** 987 (1976).