REVISTA ESPAÑOLA DE FISIOLOGIA, 45 (1), 15-20, 1989

Rat Blood and Brain Amino Acid Pattern in Short Term Experimental Diabetes

G. Atienza*, M. Aldegunde, J. A. R. Veira, A. Lorenzo** and R. Conejero**

Departamento de Fisiología
Facultad de Biología
15706 Santiago de Compostela (Spain)

(Received on April 28, 1988)

G. ATIENZA, M. ALDEGUNDE, J. A. R. VEIRA, A. LORENZO and R. CONEJERO. *Rat Blood and Brain Amino Acid Pattern in Short Term Experimental Diabetes.* Rev. esp. Fisiol., 45 (1), 15-20 1989.

Some serum and brain amino acid variations ocurring in animals with short term streptozotocin-diabetes (24 h) are studied in this work. Diabetic animals showed an increase in serum of the three branched-chain amino acids as well as an increase in free tryptophan, besides a decrease in total serum tryptophan and in the tryptophan/competitor amino acids ratio. In brain, the three branched-chain amino acids increased, but there were no variation in whole brain tryptophan. Nevertheless, by studying levels of tryptophan in different brain regions, an increase in medulla-pons was recorded. This circumstance could be explained by the increase in free serum tryptophan levels, in agreement with several authors who assign this reason for brain tryptophan.

Key words: Diabetes, Streptozotocin, Branched-chain amino acids, Tryptophan.

The availability of essential amino acids in brain increasingly appears as an important mechanism by which many pathways of cerebral metabolism are regulated. Brain uptake of the large neutral amino acids (tryptophan, tyrosine, phenylalanine, leucine, isoleucine and valine) occurs via a single saturable transport system (23), located at the level of the blood-brain barrier. Transport kinetics follow the rules of competitive inhibition (24) and therefore the transport of any of the above mentioned amino acids depends both on the serum levels of the single amino acid and on the blood concentration of its competitors for brain entry.

In experimental diabetes induced by streptozotocin (STZ), an amino acid pattern characterized by high levels of serum and brain branched-chain amino acids (BCAA), (valine, leucine and isoleucine) appears, associated to a diminution of the brain concentrations of aromatic amino acids (tryptophan, tyrosine and phenylalanine) (4, 7). Amino acid supply in brain has been shown to influence the rate of synthesis of several putative neuro-

* To whom correspondence should be addressed.
** C. S. «Juan Canalejo». 15080 La Coruña (Spain).

transmitters (11, 30, 31). Due to this, the decrease in the brain levels of tryptophan found in experimental diabetes (6, 17, 18) is very important, so that it could be the origin of serotonin metabolism alterations. Nevertheless, the above mentioned results originate from long term studies. No work on short time variations has been done as yet. The present work pretends, therefore to study the amino acid, serum and brain variations occurring in the streptozotocin diabetes after twenty four hours. A special attention to the tryptophan, precursor of serotonin, was given.

Materials and Methods

Male Wistar rats weighing 250-300 g were housed in groups of 5 per cage under a controlled temperature (22 ± 1 °C), with a schedule of 12 h light and 12 h darkness (light on at 08:00 h). Food (standard laboratory, Purina chow) and water were provided *ad libitum*. The animals were fasted overnight and diabetes was induced by a single injection of Streptozotocin (STZ) (Sigma) (75 mg/kg b.w., i.p.) dissolved in citrate buffer pH 4.5. Control animals received injection of the buffer only. This method for producing diabetes is standard (25), by which streptozotocin produces diabetes by destruction of the insulin-secreting β-cells of the pancreas (1, 15).

Rats were killed by decapitation 24 h after STZ injection. Blood was collected from the cervical trunk and used for the determination of different biochemical parameters (glucose, osmolarity, urea, fructosamine, sodium, potassium, triglycerides, cholesterol and NEFA). Serum fructosamine concentrations were determined with commercial reagents and automated centrifugal analyzer (F. Hoffmann-LaRoche, Basel). Serum osmolarity was determined using a Knauer osmometer and sodium and potassium by a selective electrode system (Electrolite, Beckman). All other routine chemistry tests were done on a model 704 Hitachi autoanalyzer. For serum amino acids determination, blood was immediately deproteinized with 2M ice-cold sulfosalicylic acid solution, and the precipitated proteins were removed by centrifugation. The supernatant was used for amino acids determination on a Technicon TSM amino acid analyzer by the method of CONEJERO *et al.* (5). Free (ultrafiltrable serum) and total (acid-soluble) serum tryptophan concentrations were determined by a modification (2) of the fluorometric method of DENCKLA and DEWEY (10). Like -wise liver tryptophan concentration was determined by using the same technique (2); to this end, a sample of liver was removed at room temperature, weighed and homogenized in 10 % trichloroacetic acid (TCA).

After decapitation, the brain was quickly removed from the skull, bisected midsagitally and frozen immediately on dry ice. One hemisphere was weighed and homogenized in a solution of cold 0.12 M sulfosalicylic acid, and amino acids determined as above. Another brain hemisphere was dissected into the following regions: medulla-pons, midbrain, hypothalamus, hippocampus, striatum and cortex, using the method of MCEWEN and PFAFF (21). After weighing, each region was homogenized into acidified butanol and employed for the tryptophan determination according to the method of BLOXAM and WARREN (2) with minor modifications.

Statistical analysis of results were performed by using the Student'*t* Test.

Results

The animals treated with STZ present an increase in the levels of glucose, urea, triglycerides, cholesterol, potassium and NEFA 24 h after induction. As well as a decrease in sodium concentration without

Table I. *Levels of different biochemical parameters in control and diabetic rats.*
Values are means ± SD. Number of rats per group, 8. Significance levels of differences from controls: *P < 0.050; **P < 0.025; ***P <0.001.

Parameters	Control	Diabetic
Urea (mg/dl)	36.7 ± 1.2	47.6 ± 2.1***
Glucose (mg/dl)	146 ± 2.4	539 ± 17***
Fructosamine (mmol/l)	1.45 ± 0.1	1.22 ± 0.1
Triglycerides (mg/dl)	157 ± 19	640 ± 34***
Sodium (mEq/l)	143 ± 0.3	135 ± 0.8***
Potassium (mEq/l)	7.63 ± 0.1	8.54 ± 0.3**
Osmolarity (mOsm/l)	296 ± 3.5	307 ± 5.1
Cholesterol (mg/dl)	64.9 ± 4.1	78.9 ± 4.8*
N.E.F.A. (mEq/l)	389 ± 20	2294 ± 110***

osmolarity and fructosamine variations (table I).

Important increases were observed (table II) in serum concentrations of the three branched-chain amino acids and in tyrosine, whereas glutamine, glycine and arginine decreased. Nevertheless, branched-chain amino acids increased while tyrosine and phenylalanine showed an important fall in brain.

With regard to tryptophan concentrations (table III), a decreased level of total serum tryptophan has been found together with a large decrease in albumin-bound tryptophan. In addition, an increase in free serum tryptophan with no variations in liver tryptophan were noted. Regarding brain, there were no significant changes in tryptophan, taking the whole brain into account, but there were variations if regions were taken into consider-

Table II. *Serum and brain amino acid levels in control and diabetic rats.*
Values are means ± SD. Number of rats per group, 8. Serum amino acids are expressed as μmol/l and brain amino acids in nmol/g. ND, not detectable. Significance levels of differences from controls: *p < 0.050; **p < 0.025; ***p < 0.001.

Amino Acids	Serum		Brain	
	Control	Diabetic	Control	Diabetic
Aspartate	30.0 ± 2.4	25.0 ± 1.5	2171 ± 93	2170 ± 94
Threonine	221 ± 14	239 ± 14	511 ± 18	493 ± 39
Serine	195 ± 17	190 ± 12	1127 ± 62	1028 ± 77
Glutamate	183 ± 11	192 ± 10	9089 ± 337	9362 ± 440
Glutamine	526 ± 30	255 ± 32***	5429 ± 383	3375 ± 296***
Glycine	280 ± 21	215 ± 10**	657 ± 46	545 ± 27**
Alanine	356 ± 26	329 ± 23	417 ± 32	343 ± 23*
Methionine	39.4 ± 3.5	38.7 ± 3.3	30.8 ± 2.2	26.0 ± 2.0
Valine	111 ± 5.2	298 ± 27***	45.7 ± 2.1	79.2 ± 4.2***
Leucine	109 ± 5.5	273 ± 22***	68.2 ± 5.3	111 ± 7.7***
Isoleucine	72.8 ± 3.5	199 ± 17***	30.5 ± 1.6	52.4 ± 3.9***
Tyrosine	65.5 ± 5.3	93.8 ± 8.4**	56.6 ± 2.4	45.6 ± 3.6**
Phenylalanine	60.1 ± 4.1	63.2 ± 3.7	42.4 ± 2.5	29.3 ± 3.0**
Lysine	244 ± 29	191 ± 26	128 ± 4.4	107 ± 7.1**
Histidine	57.6 ± 7.0	71.1 ± 7.7	61.7 ± 4.9	52.1 ± 1.4*
Arginine	151 ± 13	111 ± 8.7**	130 ± 14	137 ± 14
Asparagine	76.1 ± 7.6	84.2 ± 4.0	ND	ND
Taurine	336 ± 22	376 ± 24	5399 ± 532	5350 ± 320
Cystine	21.5 ± 2.6	22.8 ± 3.4	ND	ND

Table III. *Serum, liver and brain tryptophan concentrations in control and diabetic rats.*
Values are means ± SD. In parentheses are number of rats. Serum concentrations are expressed as µmol/l and liver and brain concentrations in nmol/g. Tryptophan ratio: serum total tryptophan/Σ valine + + leucine + isoleucine + tyrosine + phenylalanine. Significance levels of differences from controls: $^{*}P < 0.025$; $^{**}P < 0.001$.

	Control (8)	Diabetic (12)
SERUM TRYPTOPHAN		
Free	11.2 ± 1.4	22.6 ± 1.8**
Total	232 ± 21	120 ± 19**
Bound	220 ± 22	96.9 ± 20**
Tryptophan ratio	0.58 ± 0.1	0.11 ± 0.02**
LIVER TRYPTOPHAN	73.2 ± 6.6	60.4 ± 6.0
BRAIN TRYPTOPHAN		
Whole brain	10.0 ± 0.7	10.8 ± 0.3
Medulla-pons	11.5 ± 0.9	14.7 ± 0.5*
Midbrain	15.5 ± 1.4	18.1 ± 0.9
Hypothalamus	28.3 ± 1.7	32.0 ± 2.4
Hippocampus	15.3 ± 0.8	17.2 ± 0.9
Striatum	17.4 ± 1.1	19.1 ± 0.7
Cortex	4.6 ± 0.5	4.8 ± 0.3

ation since at medulla-pons tryptophan increased.

Discussion

Different investigators have shown that treatment of animals with STZ induced a similar process to the type I (insulin-dependent) diabetes mellitus (14, 16, 26), so that this experimental model has been widely used in the study of this illness.

Regarding the changes ocurred in some biochemical parameters at twenty four hours from the beginning of the experimental diabetes, it may be stated that diabetes begins quickly, because there is a great increase in values of glucose, urea, triglycerides, cholesterol, potassium and NEFA, as well as an important decline of sodium. The absence of variation in the osmolarity and fructosamine was expected as these parameters can only be changed at long term.

Serum and brain levels of some large neutral amino acids are disturbed in short term STZ-diabetes, according to the present results. Serum leucine, isoleucine and valine levels are significantly increased. This may be due to secretion and insulin action absence, so that amino acid flow from muscle to blood would be increased (4). Although the present findings are obtained 24 h after diabetes inception, they agree with results from other authors who found high levels of serum BCAA in long term STZ-diabetes (4, 7, 32). A significant increase in the three BCAA was also found in brain, which agrees with the increased brain uptake of these amino acids due to their increased circulating levels. These results are in agreement with those of CRANDALL and FERNSTROM (7) and BROSNAN *et al.* (4), but they do not agree with those of ZIPARO *et al.* (32), who did not find any significant changes in BCAA.

With regard to the aromatic amino acids tyrosine and phenylalanine, tyrosine levels in diabetic rats serum is significant increased but phenylalanine level is the same. However, both tyrosine and phenylalanine decreased in brain. Several authors (4, 7, 32) did not find any significant changes in serum concentrations of these two amino acids in long term STZ-diabetes studies, while the few existing works about brain, show contradictory results. CURZON and FERNANDO (8), did not appreciate any significant changes in any of them; ZIPARO *et al.* (32) only found decreased tyrosine, while BROSNAN *et al.* (4) and CRANDALL and FERNSTROM (7) found a decrease in the two amino acids, tyrosine and phenylalanine.

With respect to tryptophan, the increase in serum free concentration could be explained by the great increase in the NEFA levels. NEFA is known to compete with tryptophan for binding to albumin (22). Thus, an NEFA increase would lead to a larger binding to albumin;

so that tryptophan would diminish in its bound form, while increasing in its free form. Otherwise, liver tryptophan shows a decrease in its concentration which could be related to an increase in liver tryptophan pyrrolase activity, which increases in STZ-diabetes (27).

It has been shown that in long term STZ-diabetes there exists a decrease in brain tryptophan concentration (4, 6, 7, 17, 18). Due to the fact that the half-life of STZ in experimental animals is relatively short (28, 29) and that there is no evidence of the existence of binding sites for STZ in brain, it is not likely that changes in brain tryptophan could be made directly by the same streptozotocin. Some authors (11, 12) have indicated that brain typtophan concentrations depend on total serum tryptophan or on the ratio of total serum tryptophan to the sum of neutral amino acids which compete with tryptophan for entry into the brain. Taking this fact into account as well as the diminution of total serum tryptophan and the serum ratio found in this experiment, a decrease in brain tryptophan levels would be expected. Nevertheless, not only were no changes found in this parameter, but a significant increase was found in medulla-pons by analyzing it in different brain regions. This circumstance could be explained by the increase in free serum tryptophan levels, in agreement with other authors (3, 9, 13, 19, 20) who think that this is the reason for brain tryptophan levels.

Acknowledgement

This work was supported in part by Grant 87/1463 from the «Fondo de Investigaciones Sanitarias (F.I.S.)» (Spain).

Resumen

Se estudian las variaciones aminoacídicas séricas y cerebrales que aparecen en ratas con diabetes experimental a las 24 h de su inducción por estreptozotocina. Los animales diabéticos muestran importantes alteraciones en la bioquímica sanguínea, además de un incremento en las concentraciones séricas de aminoácidos ramificados y del triptófano libre, junto a un descenso en el triptófano sérico total y en el cociente triptófano/aminoácidos competidores. En el cerebro, se incrementan los tres aminoácidos ramificados, sin apreciarse ninguna variación en la concentración cerebral total de triptófano. Sin embargo, al estudiar su concentración cerebral en diferentes regiones, se halla incrementado en la médula puente. Este hallazgo puede explicarse por el incremento de los niveles del triptófano sérico libre, apoyando la hipótesis de diferentes autores de que éste es el principal factor en el control del triptófano cerebral.

Palabras clave: Diabetes, Estreptozotocina, Aminoácidos ramificados, Triptófano.

References

1. Arison, R. N., Ciaccio, E. I., Glitzer, M. S. and Casaro, J. A.: *Diabetes*, 16, 51-56, 1967.
2. Bloxam, D. L. and Warren, W. H.: *Anal. Biochem.*, 60, 621-625, 1974.
3. Bloxam, D. L., Tricklebanck, M. D., Patel, A. J. and Curzon, G.: *J. Neurochem.*, 34, 43-49, 1980.
4. Brosnan, J. T., Man, K., Hall, D. E., Colbourne, S. A. and Brosnan, M.E.: *Am. J. Physiol.*, 244, E151-E158, 1983.
5. Conejero, R., Lorenzo, A., García, J., Latorre, R, García, C. and Martín, M.T.: *Med. Intensiva*, 8, 35-39, 1984.
6. Crandall, E. A. and Fernstrom, J. D.: *Diabetes*, 29, 460-466, 1980.
7. Crandall, E. A. and Fernstrom, J. D.: *Diabetes*, 32 222-230, 1983.
8. Curzon, G. and Fernando, J. C. R.: *Br. J. Pharmacol.*, 60, 401-408, 1977.
9. Curzon, G. and Knott, P. J.: *Br. J. Pharmacol.*, 50, 197-204, 1974.
10. Denckla, W. D. and Dewey, H. R.: *J. Lab. Clin. Med.*, 69, 160-169, 1967.
11. Fernstrom, J. D. and Wurtman, R. J.: *Science*, 178, 414-416, 1972.
12. Fernstrom, J. D., Faller, D. V. and Shabshelowitz, H.: *J. Neural. Transm.*, 36, 113-121, 1975.
13. Gessa, G. L. and Tagliamonte, A.: In «Aro-

matic Amino Acids in the Brain», (Wolstenholme, G. E. W. and Fitzsimmons, D. W., eds.), Elsevier, London, 1974, pp. 207-216.
14. Hoftiezer, V. and Carpenter, A. M.: *Diabetologia*, 9, 178-184, 1973.
15. Hoftiezer, V. and Carpenter, A. M.: *Wien Z. Inn. Med. Ihre Grenzgeb*, 52, 36-40, 1971.
16. Junod, A., Lambert, A. E., Stauffacher, W. and Renold, A. E.: *J. Clin. Invest.*, 48, 2.129-2.139, 1969.
17. Mackenzie, R. G. and Trulson, M. E.: *J. Neurochem.*, 30, 205-211, 1978.
18. Mackenzie, R. G. and Trulson, M. E.: *J. Neurochem.*, 31, 157-160, 1978.
19. Madras, B. K., Cohen, H. L., Fernstrom, J. D., Larin, F., Munro, H. N. and Wurtman, R. J.: *Nature*, 244, 34-35, 1973.
20. Madras, B. K., Cohen, H. L., Messing, R., Munro, H. N. and Wurtman, R. J.: *Metabolism*, 23, 1.107-1.116, 1974.
21. Mcewen, B. S. and Pfaff, D. W.: *Brain Res.*, 21, 1-16, 1970.
22. McMenamy, R. H. and Oncley, J. L.: *J. Biol. Chem.*, 233, 1.436-1.440, 1958.
23. Oldendorf, W. H.: *Am. J. Physiol.*, 221, 1.629-1.639, 1971.
24. Pardridge, W. M.: *J. Neurochem.*, 28, 103-108, 1977.
25. Rerup, C. C.: *Pharmacol. Rev.*, 22, 485-518, 1970.
26. Rossini, A. A., Like, A. A., Chick, W. L., Appel, M. C. and Cahill, G. F.: *Proc. Natl. Acad. Sci. U.S.A.*, 74, 2.485-2.489, 1977.
27. Sadler, E. M., Weiner, M. and Buterbaugh, G. G.: *Biochem. Arch.*, 1, 15-24, 1985.
28. Saiki, O., Negoro, S., Tsuyuguchi, I. and Yamamura, Y.: *Infec. Immun.*, 28, 127, 1980.
29. Schein, P. S., Kahn, R., Gorden, P., Wells, S. and Devita, V. T.: *Arch. Intern. Med.*, 132, 555, 1973.
30. Schwartz, J. C., Lampart, C. and Rose, C.: *J. Neurochem.*, 19, 801-810, 1972.
31. Wurtman, R. J., Larin, F., Mostafapour, S. and Fernstrom, J. D.: *Science*, 185, 183-184, 1974.
32. Ziparo, V., Castorina-Ziparo, S., James, J. H., Edelstein, E. and Fischer, J. E.: *J. Surg. Res.*, 26, 547-554, 1979.