CEREBRAL AMINO ACIDS IN FLUOROACETATE-POISONED, ANAESTHETISED AND HYPOGLYCAEMIC RATS

by

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Previous results have shown that during insulin hypoglycaemia and thiopentone anaesthesia there is a reduction in the concentration of free glutamic acid in the brain of the rat (DAWSON[1,2]). In these experiments the fall in the cerebral glutamic acid level occurring in hypoglycaemia was found to be unconnected with the general hypoaminoacidoma of the blood, and the suggestion was made that in a hypoglycaemic emergency the brain can oxidise part of its glutamic acid content. The reduction of the glutamic acid level in the hypoglycaemic brain has been confirmed by CRAVIOTO, MASSIEU AND ISOQUIERDO[3] using chromatographic methods, and they have made the important additional observation that the aspartic acid level is raised. If these two changes are interrelated it would suggest that the glutamic acid is partly donating its carbon skeleton to the tricarboxylic acid cycle by transamination.

It is therefore possible that agents which interfere with the normal utilization of glucose by the brain can cause secondary changes in the concentrations of the free amino acids in the tissue. In this investigation the levels of certain cerebral amino acids which are closely related to glutamic acid in tissue metabolism have been measured during fluoroacetate poisoning, insulin hypoglycaemia and thiopentone anaesthesia. The administration of fluoroacetate is now thought to cause an accumulation of fluorocitrate in the tissue which specifically blocks the tricarboxylic acid cycle by inhibiting aconitase (PETERS[4]).

METHODS

Female albino rats (80–120 g) starved 4–12 h were used throughout the investigation. These were decapitated and the heads immediately frozen in liquid oxygen. This freezing technique was considered necessary because of the presence of proteolytic enzymes in rat brain tissue which are active at a physiological pH (ANSELL, WILLIAMS AND RICHTER[5]). The frozen brain tissue was dissected and powdered, and a solution of the amino acids suitable for chromatography was prepared by the method of AWAPARA[6], the bulk of the proteins being removed by heat coagulation and precipitation with 60% v/v ethanol.

Chromatography. Chromatograms of the amino acids from 40 mg brain were prepared using a descending flow of phenol saturated with a 0.1% solution of NH_3 in water. They were dried free from water in a current of air and the phenol thoroughly extracted by washing three times in ether. The papers were sprayed on both sides with 0.1% w/v ninhydrin in water-saturated butanol, heated to 100° for 7 minutes and left overnight. Amino acid N determinations by the method of ROBERTS AND FRANKEL[7] were carried out on four of the ninhydrin-reacting spots containing aspartic acid, glutamic acid plus a little ethanolamine phosphoric acid, alanine plus glutamine, and γ-aminobutyric acid. It has been shown that in this method the colour yields from differing concentrations

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References p. 552.

of an amino acid obey the Beer-Lambert Law (ANSELL[8]). The appropriate standards were used, glutamine being employed as a standard for the glutamine plus alanine spot. A composite spot containing serine, glycine, and taurine was not investigated as it tended to be diffuse and often merged into the glutamic acid spot. The identities of the amino acids whose concentrations were found to change were verified on two-dimensional chromatograms with phenol/NH_3 and "collidine" solvents.

RESULTS

The amino acid levels in the brain of an experimental animal were directly compared with the levels in a control animal killed at the same time. In this way the effect of factors such as temperature diet and exercise, which are known to influence the concentrations of cerebral amino acids (THOMPSON *et al.*[9], WILLIAMS *et al.*[10]) were reduced to a minimum, and comparatively small changes could be detected. Changes in the amino acid levels in the three experimental groups are all recorded in Table I.

TABLE I

CHANGES IN THE CONCENTRATIONS OF CEREBRAL AMINO ACIDS DURING FLUOROACETATE POISONING, THIOPENTONE ANAESTHESIA, AND INSULIN HYPOGLYCAEMIA

Results are recorded as the change in μg of amino-acid N/g of brain tissue

	Time after injection mins.	*Glutamic acid*	*Aspartic acid*	*Glutamine + alanine*	*γ-Amino butyric acid*
Normal range (μg/N/g)		(152–179)	(43–76)	(49–88)	(24–39)
Fluoroacetate poisoned (8–15 mg/kilo, i.p.)					
Hypoactive	65	−22	+ 3	—	− 2
Immobile	85	−14	− 1	—	+ 3
Immobile	55	−32	−12	+27	+ 5
Immobile	95	−36	− 6	+28	+ 2
Head retraction	80	−29	−13	+18	+ 4
Immediately after convulsion	50	−20	−10	+21	+ 2
Immediately after convulsion	60	−16	−11	+43	+ 3
Mean change	—	−24	− 7	+27	+ 2
P		< 0.01	< 0.03	< 0.01	
Anaesthetized (50 mg/kilo thiopentone i.p.)					
Light (corneal reflex)	40	−21	+10	+ 5	− 5
Deep	75	−41	+ 8	+11	+ 2
Deep	115	−46	+ 9	+ 9	− 1
Deep	45	−16	+15	+10	− 6
Very deep	50	−35	+23	− 4	+ 2
Mean change	—	−32	+13	+ 6	− 2
P		< 0.01	< 0.01		
Hypoglycaemic (50–100 units/kilo insulin i.p.)					
Slightly depressed	105	−20	+19	—	− 4
Depressed	70	—	+21	−10	− 6
Semi-coma	105	−33	+17	− 4	+ 3
Semi-coma	140	−28	+12	−11	− 8
In convulsions	115	−41	+28	−10	− 1
Mean change	—	−30	+19	− 9	− 3
P		< 0.01	< 0.01		

Fluoroacetate poisoning

A series of rats were killed at various times after they had been injected with a

References p. 552.

lethal dose of fluoroacetate (8–15 mg/kilo). These animals all showed a reduction of the cerebral glutamic acid level while in all the animals suffering from relatively severe signs of the poison there was also a small decrease in the aspartic acid concentration. The glutamine + alanine level increased markedly in the poisoned animals, while all but one animal showed a very small increase in the level of γ-amino-butyric acid.

Glutamine determination by the method of RICHTER AND DAWSON[11] gave a mean value for four of the fluoroacetate poisoned animals of 53 μg N/g while the controls had a mean level of 57 μg N/g. While the low values for the control animals indicated that some decomposition of glutamine had occurred during the deproteinising procedure, the results gave no evidence that the raised glutamine + alanine level in the fluoroacetate poisoned animals was due to glutamine accumulation, and therefore indicated the formation of alanine. This was confirmed by hydrolysing the glutamine in extracts by heating for 5 min at 100° in 2 N H_2SO_4 which was then removed with barium. Although the chromatograms prepared from such a hydrolysate were of a poor quality it was easily observed that the fluoroacetate had caused a marked accumulation of alanine in the brain. While this investigation was in progress AWAPARA[12] has reported that during fluoroacetate poisoning there is a reduction in the glutamic and aspartic acid levels in the heart, liver and kidney of the rat. Alanine was only estimated in the liver and unlike brain tissue its concentration appeared to decrease after fluoroacetate was administered.

Anaesthesia

Rats which had been anaesthetised with thiopentone for 40–100 minutes showed a reduction in the cerebral glutamic acid level and a small rise in the aspartic acid content. The slight rise in the glutamine + alanine level, although not significant, is probably a reflection of the raised glutamine content found during anaesthesia (DAWSON[2]).

Hypoglycaemia

Determinations of the cerebral amino acids in rats which were hypoglycaemic after insulin administration confirmed the fall in the glutamic acid level previously reported, while there was at the same time a rise in the aspartic acid concentration. The glutamine + alanine level was slightly lower than in the controls, while the γ-amino butyric acid level did not change significantly.

DISCUSSION

The cerebral amino acid changes recorded in fluoroacetate-poisoned animals could be explained by the hypothesis that the inhibition of aconitase causes an increase in the concentration ratio between pyruvate and oxaloacetate so that aspartic acid transaminates with pyruvic acid to form alanine. The glutamic acid disappearing may be donating α-ketoglutaric acid to the tricarboxylic acid cycle either by glutamic dehydrogenase activity or by transamination. If transamination occurs the aspartic acid produced must be further transaminated into alanine as the aspartic acid level in the brain does not increase. During hypoglycaemia the reduced glucose supply lowers the level of pyruvate in the brain (OLSEN AND KLEIN[13]). This reduction in the supply of substrate for the tricarboxylic acid cycle could cause a lowering of the concentration

References p. 552.

ratio between α-ketoglutarate and oxaloacetate, so that glutamic acid would transaminate producing aspartic acid. The reduced functional activity of the brain during barbiturate anaesthesia may also produce a reduction of the cerebral pyruvic acid level, as it is known that the lactic acid content of the brain is diminished (STONE[14]; RICHTER AND DAWSON[15]). If this were so, it may cause, in a similar manner to that postulated for the hypoglycaemic animal, glutamic acid to transaminate with oxaloacetate producing aspartic acid and donating α-ketoglutaric acid to the tricarboxylic acid cycle.

It is not possible to decide whether the glutamic acid disappearing in the conditions investigated is entirely removed by transamination or whether α-ketoglutarate is also produced through the glutamic dehydrogenase system which is known to be appreciably active in brain tissue (COPENHOWER, MCSHAN AND MYER[16]). However, there is little doubt that the carbon skeleton of the glutamic acid disappearing is being oxidised with the consequent production of energy. Cerebral mitochondria are able to use glutamic acid as a substrate for oxidative phosphorylation with an efficiency equal to that of intermediates of the tricarboxylic acid cycle (ABOOD AND GERARD[17], BRODY AND BAIN[18]). It is not suggested that in the conditions investigated the energy produced by glutamate oxidation is of major physiological significance, but it should be remembered that the energy used by the brain during these emergencies is very much lower than that by the organ during normal functional activity. It is becoming increasingly clear that the brain has a considerable number of these auxiliary buffering reactions which minimise the effects of an interference with the normal supply of energy from glucose metabolism (KETY, WOODFORD, HARMEL, FREYHAM, APPEL AND SCHMIDT[19]; GEIGER, MAGNES AND GEIGER[20]).

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SUMMARY

Changes which occur in the concentrations of certain amino acids in rat brain during fluoroacetate poisoning, insulin hypoglycaemia and thiopentone anaesthesia have been measured.

Fluoroacetate poisoning causes a reduction in the cerebral glutamic acid and aspartic acid levels, and an increase in the alanine concentration.

Both insulin hypoglycaemia and thiopentone anaesthesia produce a reduced glutamic acid content and a rise in the aspartic acid level.

RÉSUMÉ

L'auteur a étudié les modifications de la concentration de certains aminoacides dans le cerveau du rat au cours de l'empoisonnement par le fluoroacétate, de l'hypoglycémie insulinique, et de l'anesthésie par la thiopentone.

L'empoisonnement par le fluoroacétate entraîne une réduction de la teneur du cerveau en acides glutamique et aspartique, et une augmentation de la concentration en alanine.

L'hypoglycémie insulinique et l'anesthésie par la thiopentone s'accompagnent toutes les deux d'une diminution de la teneur en acide glutamique et d'une augmentation de la teneur en acide aspartique.

ZUSAMMENFASSUNG

Die Veränderungen, die in der Konzentration von gewissen Aminosäuren in den Gehirnen von Ratten bei Fluoracetatvergiftung, Insulinhypoglycaemie und Thiopentonanaesthesie auftreten, wurden gemessen.

References p. 552.

Fluoracetatvergiftung verursacht eine Erniedrigung des Glutaminsäure- und Asparaginsäurespiegels im Gehirn und ein Ansteigen der Alaninkonzentration.

Sowohl Insulinhypoglycaemie, wie Thiopentonanaesthesie erzeugen einen verminderten Glutaminsäuregehalt und ein Ansteigen des Asparaginsäurespiegels.

REFERENCES

1 R. M. C. DAWSON, *Biochem. J.*, 47 (1950) 386.
2 R. M. C. DAWSON, *Biochem. J.*, 49 (1951) 138.
3 R. O. CRAVIOTO, G. MASSIEU AND J. J. IZQUIERDO, *Proc. Soc. Exp. Biol. Med.*, 78 (1951) 856.
4 R. A. PETERS, *Proc. Roy. Soc.*, 139 (1952) 143.
5 G. B. ANSELL, R. B. WILLIAMS AND D. RICHTER, *Biochem. J.*, 50 (1952) xxix.
6 J. AWAPARA, *Arch. Biochem.*, 19 (1948) 172.
7 E. ROBERTS AND S. FRANKEL, *J. Biol. Chem.*, 187 (1950) 55.
8 G. B. ANSELL, Ph. D. Thesis, University of Wales (1952).
9 H. T. THOMPSON, P. E. SCHURR, L. M. HENDERSON AND C. A. ELVEHJEM, *J. Biol. Chem.*, 182 (1950) 47.
10 J. N. WILLIAMS, P. E. SCHURR AND C. A. ELVEHJEM, *J. Biol. Chem.*, 182 (1950) 55.
11 D. RICHTER AND R. M. C. DAWSON, *J. Biol. Chem.*, 176 (1948) 1199.
12 J. AWAPARA, *J. Biol. Chem.*, 197 (1952) 695.
13 N. S. OLSEN AND J. R. KLEIN, *Arch. Biochem.*, 13 (1947) 343.
14 W. E. STONE, *Biochem. J.*, 32 (1938) 1908.
15 D. RICHTER AND R. M. C. DAWSON, *Am. J. Physiol.*, 154 (1948) 73.
16 J. H. COPENHAVER, W. H. MCSHAN AND R. K. MEYER, *J. Biol. Chem.*, 183 (1950) 73.
17 L. G. ABOOD AND R. W. GERARD, *Am. J. Physiol.*, 168 (1952) 739.
18 T. M. BRODY AND J. A. BAIN, *J. Biol. Chem.*, 195 (1952) 685.
19 S. S. KETY, R. B. WOODFORD, M. H. HARMEL, F. A. FREYHAM, K. E. APPEL AND C. F. SCHMIDT, *Am. J. Psychiat.*, 104 (1948) 765.
20 A. GEIGER, J. MAGNES AND R. S. GEIGER, *Nature*, 170 (1952) 754.

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