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THE EFFECT OF STREPTOZOTOCIN DIABETES ON BRAIN PROTEIN SYNTHESIS IN THE RAT

Effet du diabète streptozotocique sur la synthèse des protéines du cerveau chez le rat

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SUMMARY

Young normal control rats were compared with a group made diabetic by treatment with streptozotocin and with other normal rats maintained on a restricted diet to obtain a daily body weight change similar to that of the diabetics. In diabetic rats plasma levels of Val, Ile and Leu rose and those of Asp, Thr, Ser, Gln, Gly, Tyr, Orn, Lys, His and Arg decreased, whereas brain concentrations of Leu, Arg and Orn were augmented and those of Thr and Ser reduced. Insulin treatment diminished these differences in comparison with controls values. In food-restricted normal rats plasma and brain amino acid concentrations also differed from values in normal controls but they were of different magnitude and/or direction than those of diabetics. *In vitro* ^{3}H-Leucine incorporation into proteins by brain postmitochondrial dialyzed supernatants was unaffected in both diabetic and food-restricted rats, whereas in liver preparations the same parameter was significantly reduced in both groups and insulin treatment of the diabetics decreased this difference. Results indicate that brain amino acid concentrations in diabetic aminals are a secondary consequence of their circulating levels and of potential modifications of brain amino acid metabolism other than protein synthesis, which is unaffected.

Key words : Streptozotocin diabetes. Amino acids. Brain protein synthesis. Liver protein synthesis. Insulin.

RÉSUMÉ

De jeunes rats normaux (contrôles) ont été comparés avec un groupe de rats rendus diabétiques par traitement par streptozotocine et avec d'autres rats normaux soumis à un régime restrictif de façon à obtenir des modifications pondérales quotidiennes analogues à celles rencontrées chez les diabétiques. Chez les rats diabétiques, les taux plasmatiques de Val, Ile, Leu s'élèvent et ceux d'Asp, Thr, Ser, Gln, Gly, Tyr, Orn, Lys, His et Arg diminuent alors que les concentrations cérébrales de Leu, Arg et Orn augmentent et celles de Thr et Ser sont diminuées. Le traitement insulinique diminue les différences constatées avec les valeurs de contrôle. Chez les rats normaux soumis à un régime restrictif, les concentrations plasmatiques et cérébrales d'acides aminés diffèrent également de celles des contrôles normaux, elles diffèrent de celles des diabétiques par leurs valeurs et/ou leurs signes. L'incorporation de leucine tritiée *in vitro* dans les protéines en provenance du surnageant dialysé postmitochondrial de cerveau n'est pas modifié chez les diabétiques ou les rats soumis au régime restrictif, alors que les préparations similaires faites à partir du foie étaient diminuées de façon significative dans ces deux groupes. Dans ce dernier cas, le traitement insulinique diminuait la différence chez les diabétiques. Ces résultats indiquent que les concentrations cérébrales en acides aminés de l'animal diabétique sont secondaires aux taux circulants de ces acides aminés. Elles peuvent aussi être secondaires aux modifications potentielles du métabolisme des acides aminés cérébraux autres que celles concernant la synthèse protéique qui, elle, n'est pas modifiée.

Mots clés : Diabète streptozotocique. Acides aminés. Synthèse protéique cérébrale. Synthèse protéique hépatique. Insuline.

Abbreviations used : PMS : postmitochondrial supernatant ; TCA : trichloroacetic acid.

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Diabetes is known to affect protein synthesis in the rat. Skeletal muscle has been consistently reported to reduce protein synthesis in diabetic animals, this effect being restored by insulin treatment (1-5). Similar changes are known to occur in the heart (1). Changes are less clear in the liver where

protein synthesis has been reported as either unchanged (6) or impaired (7-9) and insulin has been shown not to affect (6) or to enhance it (7-10). No studies have been reported concerning protein synthesis in the brain of diabetic animals. Amino acids fluxes are altered in all these tissues in streptozotocin-diabetic rats (11), the brain being specially affected by augmented extraction of branched-chain amino acids (11, 12) and decreased concentration of a number of neutral amino acids including tryptophan (11, 13, 14) which are transported across the blood-brain barrier by the same transporter as the branched-chain amino acids (15). Circulating concentrations of these branched-chain amino acids are greatly elevated in diabetic rats (11, 16) and a competition mechanism decreases the entry of other amino acids across the blood-brain-barrier.

We have previously shown in the rat that tryptophan overload produces a decrease in brain branched-chain amino acids (17) demonstrating a competitive relationship among these amino acids for their brain uptake. Although circulating amino acid imbalance may (18, 19) or may not (17, 20, 21) interfere with brain protein synthesis, this question should be examined in diabetes. We therefore measured plasma and brain amino acid concentration and brain protein synthesis using an *in vitro* free mitochondrial system (22), using streptozotocin diabetic rats with or without insulin treatment, animals food-restricted to retain the same body weight change as the diabetics, and controls fed «ad libitum» to correct any effects produced by the catabolic state of the diabetics. Due to the uncertainties concerning liver protein synthesis in diabetic animals, the study was extended to determine this parameter.

MATERIAL AND METHODS

Male Wistar rats from our colony weighing 120 ± 19 g were divided into four groups of similar body weight, maintained in collective cages in a temperature (22 ± 1 °C) and light (from 7.00 to 19.00 h) controlled room, and fed purina «ad libitum». One group was made diabetic after an overnight fast by a single intraperitoneal injection of streptozotocin (the Upjohn Co. Michigan) (75 mg/Kg) freshly dissolved in citrate buffer pH 4.5. Another group received the same treatment and from the seventh day following the initial administration of streptozotocin they were subcutaneously injected daily with monocomponent porcine insulin (Actrapid, from Novo Industries A/S Copenhague, Denmark) (2 UI/100 g), the last injection being given 1 h before sacrifice. A third group (food restricted controls) received no treatment but food was limited to obtain a mean daily body weight similar to that of the diabetics. A fourth group of normal controls received no treatment and was fed *ad libitum*. All rats were killed by decapitation without anesthesia 22 days after onset of the experiment. Blood was collected into heparinized, chilled tubes and plasma samples were stored at −20 °C until processing. Brains and livers were immediately excised for protein synthesis determination on the same day and aliquots of brain homogenates were stored at −20 °C for amino acid analysis. Plasma aliquots were deproteinized (23) for glucose (24) and ketone body (β-hydroxybutyrate and acetoacetate) (25) determinations by enzymatic procedures. Insulin was assayed (26) in other plasma aliquots with a rat insulin radioimmunoassay kit generously provided by Novo Industries A/S (Denmark). For amino acid determination, aliquots of plasma and brain homogenates were deproteinized with 10 % sulphosalycilic acid made in 0.1 N HCl. After centrifugation, supernatants were brought to pH 2.2 with OHLi and analyzed in a Beckman 121-MB amino acid autoanalyzer (27).

Protein synthesis in brain and liver was determined by the incorporation of ^{3}H-leucine in postmitochondrial fractions, as described previously (22). Tissues were homogenized (1:4 w/v) in a 50 mM Tris-HCl pH 7.5 buffer containing 25 mM KCl, 1 mM dithiothreitol, and 0.35 M RNase-free sucrose. After centrifugation for 15 min at 16,000 g, 0.2 ml aliquots of the postmitochondrial supernatant (PMS) were brought to a final volume of 0.3 ml containing : 0.6 mM ATP, 0.6 mM GTP, 12 mM creatine phosphate, 0.02 mg creatine kinase, and 3 mM $MgCl_2$. Then 5 μCi of ^{3}H-leucine (specific activity 47 Ci/mmol) was added at the onset of a 15 min incubation at 37 °C. The reaction was terminated by transfer to an ice bath and immediate addition of a 3 ml of cold 10 % trichloroacetic acid (TCA). Radioactivity incorporated into proteins was determined in the TCA precipitate as previously described (22) and the protein concentration was determined (28) using bovine serum albumin as standard.

RESULTS

Animals studied

Table I shows some characteristics of the experimental animals. Initial body weight was similar in all groups but on experimental day 22, it was significantly lower in the food-restricted and diabetic animals than in the group of normal animals. Insulin treatment restored body weight in diabetic animals almost to normal values. Liver weight in the groups changed similarly to body weight although values were lower in food-restricted animals than in diabetics. Brain weight remained stable among the groups except for a reduction in the diabetics. Plasma glucose concentration was significantly lower in food-restricted rats and greatly augmented in diabetic compared with normal animals ; insulin treatment reduced glycemia in diabetics although their values were still above normal. The plasma β-hydroxybutyrate level was greatly augmented in food-restricted, diabetic, and diabetic insulin treated rats compared with normals whereas the acetoacetate level was enhanced only in diabetics. Plasma radioimmunoassayed-insulin was significantly lower in food-restricted and diabetic rats than in normals and insulin treatment of diabetics produced a marked increase of this parameter compared with all other groups *(table I)*.

TABLE I. — *Characteristics of the animals studied*

	Normal (6)	*Food-restricted (6)*	*Diabetic (6)*	*Diabetic + Insulin (6)*
Weight (g)				
Initial body weight	118 ± 6	119 ± 6	120 ± 6	119 ± 6
Final body weight	189 ± 8	122 ± 8***	115 ± 8***	141 ± 7**
Liver	8.85 ± 0.75	4.00 ± 0.34***	5.15 ± 0.50**	7.59 ± 0.36
Brain	1.25 ± 0.02	1.20 ± 0.02	1.12 ± 0.02***	1.21 ± 0.05
Plasma				
Glucose (mg/dl)	120 ± 5	100 ± 4*	525 ± 19***	166 ± 13*
ß-hydroxybutyrate (µmole/dl)	1.7 ± 0.4	13.7 ± 0.9***	16.2 ± 3.8***	15.3 ± 4.6***
Acetoacetate (µmole/dl)	0.9 ± 0.4	0.9 ± 0.5	2.1 ± 0.5	0.6 ± 0.2
RIA-insulin (µU/ml)	49 ± 6	18 ± 5**	12 ± 3***	168 ± 6***

All animals were sacrificed on day 22 (day 0 = day prior streptozotocin treatment to the diabetics or saline to the controls). Diabetic + Insulin rats received a daily s.c. injection of 2 IU/100 g body wt of monocomponent porcine insulin (Novo Industries A/S Denmark) from day 7 and were sacrificed 1 h after the last injection. Values are mean ± SEM. Comparisons with normal group : * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TA[illegible] *asma amino acids levels in normal, food-restricted, diabetic and diabetic plus insulin rats.* (nmoles/ml)

Amino acid	*Normal*	*Food-restricted*	*Diabetic*	*Diabetic + Insulin*
Gluconeogenic				
Aspartic acid	56 ± 2	52 ± 4	42 ± 8***	58 ± 3
Threonine	490 ± 23	456 ± 35	307 ± 52*	267 ± 13***
Serine	353 ± 19	449 ± 24*	220 ± 34*	267 ± 20*
Glutamic acid	149 ± 6	139 ± 8	133 ± 9	186 ± 18
Glutamine	882 ± 14	771 ± 81	499 ± 35***	670 ± 80*
Glycine	510 ± 30	573 ± 25	326 ± 17***	339 ± 35*
Alanine	859 ± 39	1 157 ± 64**	806 ± 92	812 ± 12
Neutral				
Valine	303 ± 8	396 ± 38*	477 ± 47*	305 ± 9
Isoleucine	123 ± 6	248 ± 111	169 ± 10**	116 ± 3
Leucine	244 ± 12	318 ± 37	375 ± 40**	258 ± 15
Tyrosine	139 ± 7	141 ± 6	95 ± 8**	100 ± 18
Phenylalanine	82 ± 2	112 ± 7**	83 ± 4	81 ± 5
Tryptophan	134 ± 9	165 ± 3*	137 ± 11	114 ± 8
Basic				
Ornithine	82 ± 3	111 ± 6**	57 ± 7*	74 ± 8
Lysine	508 ± 68	333 ± 13	180 ± 24**	219 ± 21*
Histidine	91 ± 5	104 ± 5	55 ± 4***	58 ± 5**
Arginine	293 ± 25	255 ± 8	151 ± 13***	190 ± 32*
Total AA	6007	6585	4834	4835

Average ± SEM of at least 4 determinations. Comparisons with normal group : * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Plasma and brain amino acid concentrations.

Plasma amino acid levels in normal, food-restricted, diabetic and diabetic insulin treated rats are shown in *table II*. Values in normal rats were similar to those previously reported (29). When compared to these normal values, plasma from food-restricted rats had significantly higher concentrations of serine, alanine, valine, phenylalanine, tryptophan, and ornithine whereas plasma from diabetics showed significantly higher amounts of valine, isoleucine, and leucine and significantly less aspartate, threonine, serine, glutamine, glycine, tyrosine, ornithine, lysine, histidine, and arginine. These changes in plasma amino acid levels in diabetic rats were similar to those previously detected in the arterial blood of animals studied under similar conditions (11). Insulin treatment of the diabetic rats eliminated the differences between diabetic and normal plasma levels of aspartate, valine, isoleucine,

leucine, tyrosine, and ornithine were increased whereas other levels remained only slightly reduced. Concentrations of free amino acids in whole brains from the four experimental groups are shown in *table III*. Values found in normal rats were very similar to those previously reported (11, 17). In the brain of food-restricted rats, concentrations of serine, glycine, isoleucine, and lysine were significantly increased compared with controls whereas in the brain of diabetics, concentrations of threonine and serine were significantly decreased and those of leucine, arginine and ornithine were significantly augmented. In insulin treated diabetics, brain concentrations of threonine was significantly decreased while glycine and leucine levels were increased compared with the normals. With this technique it was not possible to determine brain concentrations of either valine or tryptophan. The fact that daily insulin injections did not restore all the parameters that are modified in streptozotocin diabetic rats is likely a consequence of the fact that circulating insulin levels due to the treatment do not mimic those obtained by endogenous insulin in normal rats.

In vitro protein synthesis in brain and liver

Brain and liver protein synthesis was estimated by the *in vitro* incorporation of ^{3}H-leucine into proteins by PMS preparations. To appreciate differences produced by potential changes in tracer specific activities due to group differences in the free amino acid pools, PMS aliquots were incubated with or without prior dialysis against the same buffer. Results are summarized in *table IV*. When experiments were performed without PMS dialysis, incorporation of ^{3}H-leucine into proteins was the same in normal and food-restricted rat brains whereas it was significantly decreased in diabetics and increased in insulin treated diabetics. These group differences disappeared when incubations were performed after dialysis of the preparations *(table IV)*. In the liver of

TABLE III. — *Amino acid levels in brain of normal, food restricted, diabetic and diabetic plus insulin rats.* (nmoles/g)

Amino acid	*Normal*	*Food-restricted*	*Diabetic*	*Diabetic + Insulin*
Taurine	3414 ± 236	3688 ± 347	4563 ± 490	3946 ± 453
Aspartate	2441 ± 73	2426 ± 85	2272 ± 108	2151 ± 138
Threonine	796 ± 65	894 ± 101	400 ± 47**	378 ± 42**
Serine	1121 ± 46	1363 ± 15**	920 ± 20*	978 ± 89
Glutamate	8260 ± 199	7927 ± 213	7720 ± 348	6951 ± 327
Glutamine	2375 ± 160	1744 ± 169*	2054 ± 157	1779 ± 238
Glycine	610 ± 25	857 ± 67*	675 ± 32	714 ± 15*
Alanine	496 ± 13	517 ± 36	431 ± 106	503 ± 12
Cystine	258 ± 53	194 ± 42	259 ± 62	157 ± 38
Methionine	22 ± 2	29 ± 2	35 ± 8	26 ± 6
Isoleucine	29 ± 6	67 ± 12*	39 ± 4	48 ± 3
Leucine	76 ± 6	110 ± 12	123 ± 4***	124 ± 7**
Tyrosine	63 ± 9	44 ± 9	48 ± 14	43 ± 9
Phenylalanine	41 ± 6	46 ± 10	46 ± 9	67 ± 8
Ornithine	39 ± 6	44 ± 4	53 ± 4*	55 ± 7
Lysine	167 ± 13	233 ± 18*	171 ± 13	173 ± 21
Histidine	69 ± 2	64 ± 2	55 ± 4	55 ± 8
Arginine	113 ± 7	112 ± 11	166 ± 12*	129 ± 16
γ-aminobutyrate	1949 ± 106	2236 ± 76	2528 ± 151	2349 ± 156

Average ± SEM of at least 4 determinations. Comparisons with normal group : * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

TABLE IV. — *^{3}H-leucine incorporation into proteins of brain and liver postmitochondrial supernatant (PMS)*

	Normal	*Food-restricted*	*Diabetic*	*Diabetic + insulin*
Brain PMS	14,484 ± 486 (20)	15,124 ± 724 (10)	12,457 ± 450*** (19)	18,173 ± 1,550** (15)
Brain PMS after dialysis	22,998 ± 2,144 (6)	17,500 ± 1,521 (6)	19,633 ± 884 (5)	21,080 ± 1,161 (5)
Liver PMS	5,175 ± 172 (6)	2,630 ± 183*** (5)	2,216 ± 313*** (4)	3,161 ± 243*** (4)
Liver PMS after dialysis	13,960 ± 1,473 (6)	5,993 ± 742*** (5)	5,832 ± 314*** (4)	9,652 ± 1,604* (5)

Data are expressed in cpm/mg protein ± SEM. ( ) number of experiments. Comparisons with normal group : * $p < 0.05$, *** $p < 0.001$.

both food-restricted and diabetic animals there was a significant decrease of ^{3}H-leucine incorporation into proteins whether or not the preparations were subjected to dialysis *(table IV)*. In liver from insulin diabetics, ^{3}H-leucine incorporation into proteins was significantly decreased in the undialyzed preparations in comparison with normals, but this difference almost disappeared when the experiment was performed after simple dialysis.

DISCUSSION

The decreased plasma concentration of some amino acids, including the gluconeogenetic ones, and the increase in branched-chain amino acids observed in streptozotocin diabetic rats are consistent with similar changes reported in diabetic rats (11, 30) and man (31). This circulating pattern was quite different from that present in our food-restricted rats which experienced a catabolic state similar to that of the diabetics, as indicated by their similar body and liver weights and their augmented circulating levels of ketone bodies, in which several circulating amino acids were augmented while branched-chain ones were unaffected. This distinction between diabetic and food-restricted animals indicates that striking differences also exist in interorgan amino acids fluxes. It has been shown in diabetics that there is augmented release of several amino acids from skeletal muscle and augmented uptake of some of them by the liver (11). In diabetic animals the liver is known specifically to take up gluconeogenetic amino acids (32). Our present results demonstrate that protein synthesis is inhibited in the liver of the diabetic rat, in agreement with results obtained with different methodology (7-9). Previous experiments (16) indicated that it was not the streptozotocin itself that affected the amino acid levels or presumably protein biosynthesis, but rather the diabetic state per se. The increased muscle proteolysis and liver gluconeogenesis during reduced protein synthesis may explain the circulating amino acid imbalance characteristic of the diabetic. This condition could be produced by their diminished circulating level of insulin and not by their catabolic state, because insulin treatment was found to invert the amino acid imbalance and food-restricted rats showed a different pattern. In these animals, circulating amino acids, especially the gluconeogenetic ones, were either unchanged or augmented and liver protein synthesis decreased to the same level as in diabetics. Undernourished rats have also been reported to show net protein losses from skeletal muscle (33), although gluconeogenesis may remain stable due to an adaptative mechanism of enhanced fat fuel consumption, as shown by their augmented circulating ketones, which maintain glycemia close to the normal range. Brain amino acid concentrations observed in our diabetic rats are a qualitative reflection of changes in plasma levels, including an increase in branched-chain amino acids. Insulin treatment of diabetic animals had little effect on their brain amino acid levels because a few of the amino acids known to use the same carrier as the branched-chain amino acids for their uptake at the blood brain barrier (15) have decreased levels in the diabetic brain. In our animals there was no evidence of a competitive action interfering with amino acid transfer, as reported in other diabetic rat studies (11). Such an effect may require greater changes in circulating amino acid levels and reported results differing from our data may be due to the streptozotocin dosage and experimental conditions used. Although protein synthesis measured in postmitochondrial fraction may not be representative of whole tissue activities, differences found between liver and brain preparations and the response of diabetic animals in what a reduction in liver protein synthesis is concerned are consistent with expected differences according to previous studies by us and other investigators (17, 34), validating observed results. Brain protein synthesis was not found to be affected by food restriction, diabetes, or insulin treatment of the diabetic animals, specially when differences in the endogenous free amino acid pools were avoided by dialyzing the preparations. No previous studies of this parameter have been reported in diabetic animals, but present results are not surprising because brain protein synthesis in the adult rat is known to be very insensitive to food restriction (33) and we have previously shown this to be also true in conditions of intense amino acids imbalance such as those produced by tryptophan overload (17). Brain insulin concentrations and receptors have been reported not to change despite severe changes in pancreatic insulin levels (35) and since insulin stimulates protein synthesis in primary cultures of fetal rat brain cells (36) the lack of effect of streptozotocin diabetes on brain protein synthesis could be ascribed to the constancy of the insulin levels in that organ. Thus the changes in brain amino acid levels in diabetic animals are a secondary consequence of their circulating levels and of potential modifications of brain amino acid metabolism other than protein synthesis, which is unaffected.

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