Brain energy metabolism in streptozotocin-diabetes

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Regional brain glucose use was measured in rats with streptozotocin-induced diabetes (65 mg/kg intravenously) of 1 or 4 weeks duration, by using [6-¹⁴C]glucose and quantitative autoradiography. The concentrations of several metabolites were measured in plasma and brain. Results were compared with those from normal untreated rats. Glucose concentrations were increased in both plasma and brain, to similar degrees in both diabetic groups. Plasma ketone-body concentrations were 0.25, 1.0 and 3.15 μ mol/ml in the control, 1-week and 4-week groups respectively (sum of acetoacetate and 3-hydroxybutyrate). Glucose use was increased throughout the brain (differences were statistically significant in 55 of 59 brain areas) after 1 week of diabetes, with an increase of 25 % for the brain as a whole. In contrast, normal rates were found throughout the brain after 4 weeks of diabetes. None of the brain areas was affected significantly differently from the others, in either diabetic group. There was no significant loss of ¹⁴C as lactate or pyruvate during the experimental period, nor was there any indication of net production of lactate in any of the groups. Other methodological considerations that could have affected the results obtained in the diabetic rats were likewise ruled out. Because the ketone bodies are expected to supplement glucose as a metabolic fuel for the brain, our results indicate that brain energy consumption is increased during streptozotocin-diabetes.

INTRODUCTION

When circulating concentrations of ketone bodies are higher than normal, such as during uncontrolled diabetes, the brain uses these as a supplementary fuel to glucose (Owen *et al.*, 1967; Hawkins *et al.*, 1971a, 1986). Ketone bodies appear to be used by brain in proportion to their plasma concentrations (Hawkins *et al.*, 1971a). Therefore, if overall fuel consumption by the brain were to remain constant during diabetes, it would be expected that the use of glucose should decrease. Some evidence suggests that, in very severe ketotic states, normal amounts of glucose are phosphorylated, but that less is oxidized, the excess being converted into lactate (Black-shear & Alberti, 1974; Ruderman *et al.*, 1974). Whether this is true for less severe states is unknown. Furthermore, because ketone-body use by brain shows a somewhat different regional distribution from glucose use (Hawkins & Biebuyck, 1979), the effect on glucose use may vary regionally. To address these issues, we measured regional brain glucose use, as well as brain and plasma concentrations and arteriovenous differences of several relevant intermediary metabolites, in rats 1 and 4 weeks after induction of diabetes with streptozotocin.

MATERIALS AND METHODS

Rats

Male Long-Evans rats weighing 255–275 g were used. Diabetes was induced by streptozotocin injection into a lateral tail vein (65 mg/kg) while the rats were anaesthetized with halothane, 1 or 4 weeks before the experiment. The streptozotocin was dissolved in 5 mM-citrate buffer, pH 4.0. All diabetic rats showed glucosuria

(2000 mg/dl or more); a few had a trace of ketonuria (Keto-Diastix; Miles Laboratories, Elkhart, IN, U.S.A.).

Chemicals

Streptozotocin was bought from Sigma Chemical Co., St. Louis, MO, U.S.A. Enzymes and coenzymes were from Boehringer Mannheim, New York, NY, U.S.A. [6-¹⁴C]Glucose (58 mCi/mmol) was from Amersham Corp., Arlington Heights, IL, U.S.A. Other chemicals used were of the best available grade.

Metabolite assays

Metabolites in brain and plasma extracts were assayed by enzymic methods (Bergmeyer, 1974).

Experiments

The following measurements were made on separate groups of normal and diabetic rats: metabolites in plasma and brain, glucose use (preliminary measurement), glucose use (quantitative autoradiographic measurement) and arteriovenous differences across the brain of several labelled and unlabelled metabolites.

Tissue sampling and extraction

Diabetic and control rats were anaesthetized with ketamine (60 mg/kg given intraperitoneally) and immediately killed by freeze-blower (Veech *et al.*, 1973). The brain sample was stored in liquid N₂. Brain tissue was extracted by procedures described by Veech & Hawkins (1974). Metabolites were measured in the extract within 5 days. Blood was drawn from the inferior vena cava into a heparinized syringe and centrifuged to obtain the plasma. After extraction with 0.5 M-HClO₄ (10 vol.) and neutralization with KOH/K₂CO₃ (15 %/15 %, w/v), the extract was assayed for pyruvate and

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acetoacetate. Glucose, lactate and 3-hydroxybutyrate were measured on the following day.

Preliminary glucose-use experiment

In order to obtain a rapid first estimate of whole-brain glucose consumption in diabetic rats, a simplified procedure was used on normal, 1-week-diabetic and 4-week-diabetic rats (six in each group). The rat was anaesthetized by injection of ketamine (30 mg/kg, a dose that we have found not to affect brain glucose use; Davis, 1987) into a lateral tail vein. Then 1–3 min later 10 μ Ci of [6- 14 C]glucose was injected into the tail vein, and 10 min later the brain was freeze-blown (Veech *et al.*, 1973). Blood was sampled from the inferior vena cava. The glucose concentration and radioactivity were measured in neutralized HClO₄ extracts of the brain and blood samples. An index of glucose use was calculated as:

$$\frac{\text{Radioactivity in brain tissue (d.p.m./g)} - \text{background radioactivity (d.p.m./g)}}{\text{SPA} \times \text{time}}$$

where SPA = blood glucose specific radioactivity and time = 10 min. Background radioactivity is unchanged [6- 14 C]glucose, calculated as [brain glucose] $\times$ SPA.

Autoradiographic glucose-use experiments

The rats were initially anaesthetized with halothane (4%), and one femoral artery and vein were cannulated while anaesthesia was maintained with 0.5–1.5% halothane in N₂O/O₂ (7:3). The rats were placed in restrainers and allowed to recover for 1 h. Brain glucose use was measured as described by Hawkins *et al.* (1985). [6- 14 C]-Glucose (50 μ Ci) was rapidly injected, arterial blood samples were collected, and the rat was killed with 150 mg of sodium pentobarbital at 8 min. The brain was removed and prepared for quantitative autoradiography. The integral of plasma glucose specific radioactivity during the experimental period was determined by liquid-scintillation counting and glucose assay.

Eight rats were used in each diabetic group, and 15 rats in the control group.

Brain/plasma glucose ratio

This measure is required for the calculation of brain glucose content in the glucose-use estimation, and was determined in the rats sampled for metabolite assay and preliminary glucose-use measurements. The brain glucose content was very consistent in the two diabetic groups. However, because the plasma glucose concentrations varied considerably, the ratio was not constant in the diabetic rats. Therefore, instead of using a value of the brain/plasma glucose ratio for each group to calculate individual brain glucose content in individual diabetic rats (as is normally done for the glucose-use measurement), a single value for brain glucose content was used for each diabetic group:

1-week diabetic 6.12 $\pm$ 0.28 (S.E.M., n = 13) μ mol/g
4-week diabetic 6.15 $\pm$ 0.28 (S.E.M., n = 12) μ mol/g

In the normal group a brain/plasma ratio of 0.26 (Mans *et al.*, 1986) was used in the normal manner.

[Our finding that brain glucose content did not correlate with plasma concentrations when the latter were high is not entirely unexpected, when the kinetics of glucose

transport into the brain are considered (Pappenheimer & Setchell, 1973).]

Arteriovenous-difference measurements

The rats were anaesthetized and prepared as described above, except that the superior sagittal sinus was exposed and they were paralysed with tubocurarine and artificially ventilated with 0.5% halothane in N₂O/O₂ (3:1). The halothane was withdrawn and the rats were allowed to recover for 45 min, while body temperature and blood gases were maintained in the normal range. (N₂O was continued until the end of the experiment.) [6- 14 C]-Glucose was then given intravenously as in the glucose-use experiment. Blood samples (about 400 μ l) were taken simultaneously from the femoral artery and sagittal sinus (venous sample) at 5, 10, 15, 20 and 30 min. (The longer 30 min time period was used to determine whether the time course of loss of label, if any were found, was the same in the two diabetic states.) Blood from a normal donor rat (0.8 μ l) was infused into the vein after each withdrawal.

Plasma samples (50 μ l) were placed on Dowex AG-1 (formate form) columns and washed on with 50 μ l of distilled water. Neutral substances (glucose) were eluted with 5 ml of distilled water, and acidic substances (lactate and pyruvate) with 5 ml of 1 M-formic acid. 14 C radioactivity (d.p.m.) was determined in the formic acid eluate. Lactate concentrations were measured in arterial and venous plasma from each rat.

Statistical analysis

SAS procedures (Statistical Analysis Systems, Cary, NC, U.S.A.) were used for statistical analysis. Comparisons were made between the diabetic groups and the control group by using analysis of variance with the Bonferroni correction for two comparisons. Differences were taken to be significant at an overall P value of 0.05 or less.

RESULTS

Plasma metabolites

As expected, large increases were seen in glucose and ketone-body concentrations in the diabetic rats (Table 1). The rise in ketone-body concentrations was greater at 4 weeks than at 1 week, possibly reflecting a more severe

Table 1. Plasma metabolites in diabetic rats

Blood was sampled from the inferior vena cava 1 or 4 weeks after induction of diabetes with streptozotocin (65 mg/kg, intraperitoneally) or from untreated control rats. Results are given in μ mol/ml as means $\pm$ S.E.M., with the numbers of rats in parentheses: *significantly different from control value at P < 0.025.

	Control (7)	Diabetic 1 week (8)	Diabetic 4 weeks (7)
Glucose	7.39 $\pm$ 0.72	29.66 $\pm$ 1.82*	16.28 $\pm$ 1.20*
3-Hydroxybutyrate	0.09 $\pm$ 0.03	0.59 $\pm$ 0.17*	1.61 $\pm$ 0.40*
Acetoacetate	0.16 $\pm$ 0.02	0.41 $\pm$ 0.13	1.54 $\pm$ 0.40*
Pyruvate	0.12 $\pm$ 0.01	0.17 $\pm$ 0.04	0.11 $\pm$ 0.03
Lactate	1.48 $\pm$ 0.24	2.26 $\pm$ 0.25	2.19 $\pm$ 0.49

Table 2. Brain metabolites in diabetic rats

Brain was sampled by freeze-blowing 1 or 4 weeks after streptozotocin injection or in untreated control rats. Results are given as means $\pm$ S.E.M., in $\mu\text{mol/g}$ wet wt. with the numbers of rats in parentheses. (Glucose values include an additional six rats in each group from the preliminary glucose-use experiments.) *Significantly different from control value at $P < 0.025$.

	Control (7)	Diabetic 1 week (7)	Diabetic 4 weeks (6)
Glucose	2.40 ± 0.14 (13)	$6.12 \pm 0.28^*$ (13)	$6.15 \pm 0.28^*$ (12)
Glucose 6-phosphate	0.18 ± 0.01	0.20 ± 0.01	0.21 ± 0.01
Pyruvate	0.08 ± 0.01	0.11 ± 0.01	0.10 ± 0.02
Lactate	1.38 ± 0.12	1.75 ± 0.15	1.29 ± 0.21
2-Oxoglutarate	0.20 ± 0.01	0.21 ± 0.01	0.25 ± 0.02
Glutamate	11.81 ± 0.25	12.44 ± 0.32	12.47 ± 0.17
Glutamine	5.54 ± 0.28	$6.53 \pm 0.33^*$	5.24 ± 0.23
Malate	0.14 ± 0.02	0.18 ± 0.02	0.16 (2)
Aspartate	2.96 ± 0.14	3.22 ± 0.20	2.96 ± 0.21
ATP	2.83 ± 0.04	2.89 ± 0.05	2.78 ± 0.05
ADP	0.54 ± 0.01	0.56 ± 0.01	0.50 ± 0.01
Phosphocreatine	4.12 ± 0.10	$4.58 \pm 0.10^*$	$4.52 \pm 0.07^*$

Table 3. Preliminary index of brain glucose use during diabetes

Results are given as means $\pm$ S.E.M.; there were six rats in each group. The glucose use index was measured as described in the Materials and methods section: it is an index which is proportional to the actual rates of glucose use, and is therefore given without units: *significantly different from control value at $P < 0.01$.

Rats	Glucose use index
Control	0.87 ± 0.04
Diabetic 1 week	$1.08 \pm 0.04^*$
Diabetic 4 weeks	0.89 ± 0.03

state of diabetes; however, glucose concentrations were not higher at 4 weeks than at 1 week.

Brain metabolites

Glucose content in brain was elevated by 155% and 156% in the 1-week- and 4-week-diabetic groups respectively (Table 2). There was a small rise in glutamine content (18%) in the 1-week group. No other changes were found in the intermediary metabolites measured, except a small (about 10%) but statistically significant increase in phosphocreatine.

Glucose use

In the preliminary experiments the index of brain glucose use was found to be significantly higher than normal (by 24%) in the 1-week-diabetic group, but not different in the 4-week-diabetic group (Table 3). These findings in the whole brain were confirmed at the regional level by using the full autoradiographic procedure. In 55 of 59 brain areas, glucose use was significantly higher than normal in the 1-week group (Table 4). The statistically significant differences ranged from +14 to +49%. The average difference for the combined areas was +25%. The 4-week-diabetic group had normal rates of glucose use, with only one significant difference, in the ventral thalamic nucleus, which was

15% lower than normal (Table 4). Fig. 1 shows glucose use in the diabetic rats plotted against the control values. The slope of the best-fitting straight line through the 1-week values was 1.25, and was significantly different from 1. The line through the 4-week values was not significantly different from 1. In neither group did any values deviate significantly from the line, determined as described by McCulloch *et al.* (1982). Thus there were no particular trends in the results on a regional basis.

Arteriovenous differences

There were no significant arteriovenous differences for lactate in any group (normal, $-0.13 \pm 0.11 \mu\text{mol/ml}$; 1-week-diabetic, -0.04 ± 0.04 ; 4-week-diabetic, -0.13 ± 0.12 ; means $\pm$ S.E.M., $n = 6$ in each group).

The total loss of ^{14}C in acidic form (i.e. as lactate or pyruvate) from the brain during the first 8 min of the experiment (i.e. the time period used in the glucose-use experiment), was estimated to be 2.7, 3.1 and 3.4% of the total ^{14}C label accumulated in the brain during this period, in the normal, 1-week and 4-week-diabetic rats respectively (assuming cerebral blood flow to be $1 \text{ ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$).

DISCUSSION

In our study the rate of glucose use was found to be higher than normal throughout the brain at 1 week after streptozotocin injection and normal at 4 weeks. At both these times plasma glucose concentrations were elevated to values clinically relevant in diabetes, and plasma ketone bodies were significantly increased, more so at 4 weeks than at 1 week. There was no evidence of significant production of either radiolabelled or unlabelled lactate at either time. The results suggest that in this model of diabetes the brain is using greater amounts of fuel than normal and therefore appears to be in a hypermetabolic state.

During streptozotocin-diabetes (as well as other states causing ketonemia, such as starvation) influx of the ketone bodies, 3-hydroxybutyrate and acetoacetate, into the brain is increased in proportion to their plasma concentrations (Daniel *et al.*, 1971; Gjedde & Crone,

Table 4. Regional brain glucose use during diabetes

Glucose use was measured in normal control rats and 1 or 4 weeks after injection of streptozotocin (65 mg/kg) by quantitative autoradiography (see the Materials and methods section for details). Results are given in $\mu\text{mol}/\text{min}$ per g as means $\pm$ S.E.M., with the number of rats in each group given in parentheses. * $P < 0.025$ compared with control value.

	Control (15)	Diabetic 1 week (8)	Difference (%)	Diabetic 4 weeks (8)	Difference (%)
Telencephalon					
Cortex					
Olfactory	0.96 $\pm$ 0.05	1.08 $\pm$ 0.04	+13	0.86 $\pm$ 0.09	-10
Clastrum	0.96 $\pm$ 0.04	1.05 $\pm$ 0.12	+9	0.84 $\pm$ 0.08	-12
Frontal	0.97 $\pm$ 0.03	1.20 $\pm$ 0.06*	+24	1.12 $\pm$ 0.07	+15
Insular	0.93 $\pm$ 0.03	1.13 $\pm$ 0.02*	+22	0.94 $\pm$ 0.07	+1
Pyriform	0.70 $\pm$ 0.04	0.90 $\pm$ 0.05*	+29	0.73 $\pm$ 0.06	+4
Cingulate	1.02 $\pm$ 0.03	1.32 $\pm$ 0.04*	+29	1.13 $\pm$ 0.05	+10
Parietal	1.09 $\pm$ 0.03	1.33 $\pm$ 0.03*	+22	1.06 $\pm$ 0.06	-3
Auditory	1.30 $\pm$ 0.05	1.57 $\pm$ 0.04*	+21	1.29 $\pm$ 0.09	-1
Occipital	1.03 $\pm$ 0.03	1.26 $\pm$ 0.05*	+22	1.01 $\pm$ 0.07	-2
Accumbens nucleus	0.85 $\pm$ 0.03	1.03 $\pm$ 0.04*	+21	0.88 $\pm$ 0.03	+4
Caudate	0.88 $\pm$ 0.02	1.06 $\pm$ 0.05*	+20	0.86 $\pm$ 0.02	-2
Globus pallidus	0.49 $\pm$ 0.02	0.73 $\pm$ 0.04*	+49	0.46 $\pm$ 0.03	-6
Amygdala	0.71 $\pm$ 0.02	0.81 $\pm$ 0.04*	+14	0.62 $\pm$ 0.04	-13
Hippocampus					
Anterior	0.62 $\pm$ 0.03	0.79 $\pm$ 0.05*	+27	0.57 $\pm$ 0.05	-8
Posterior	0.62 $\pm$ 0.02	0.77 $\pm$ 0.04*	+24	0.54 $\pm$ 0.03	-13
Dentate gyrus	0.70 $\pm$ 0.02	0.92 $\pm$ 0.05*	+31	0.76 $\pm$ 0.04	+9
Septal nucleus					
Lateral	0.60 $\pm$ 0.02	0.71 $\pm$ 0.04*	+18	0.58 $\pm$ 0.04	-3
Medial	0.76 $\pm$ 0.03	0.94 $\pm$ 0.03*	+24	0.65 $\pm$ 0.07	-14
Diencephalon					
Habenula	1.15 $\pm$ 0.04	1.51 $\pm$ 0.05*	+31	1.23 $\pm$ 0.06	+8
Hypothalamus	0.73 $\pm$ 0.03	0.98 $\pm$ 0.04*	+34	0.68 $\pm$ 0.04	+7
Thalamus					
Anterior nucleus	1.09 $\pm$ 0.04	1.42 $\pm$ 0.04*	+30	1.12 $\pm$ 0.05	+3
Ventral nucleus	0.96 $\pm$ 0.03	1.14 $\pm$ 0.05*	+19	0.82 $\pm$ 0.05*	-15
Medial geniculate	1.21 $\pm$ 0.04	1.49 $\pm$ 0.05*	+23	1.17 $\pm$ 0.05	-3
Lateral geniculate dorsal	1.01 $\pm$ 0.03	1.21 $\pm$ 0.03*	+20	1.01 $\pm$ 0.05	0
Lateral geniculate ventral	0.83 $\pm$ 0.03	1.03 $\pm$ 0.05*	+24	0.86 $\pm$ 0.04	+4
Gelatinous nucleus	1.01 $\pm$ 0.03	1.26 $\pm$ 0.04*	+15	0.99 $\pm$ 0.04	-2
Mamillary nucleus	1.24 $\pm$ 0.05	1.36 $\pm$ 0.07	+10	1.13 $\pm$ 0.06	-9
Mesencephalon					
Substantia nigra	0.74 $\pm$.003	0.99 $\pm$ 0.04*	+34	0.83 $\pm$ 0.04	+12
Red nucleus	0.89 $\pm$ 0.03	1.09 $\pm$ 0.04*	+22	0.86 $\pm$ 0.04	-3
Oculomotor complex	0.99 $\pm$ 0.02	1.27 $\pm$ 0.04*	+28	1.06 $\pm$ 0.06	+7
Interpenduncular nucleus	1.23 $\pm$ 0.04	1.47 $\pm$ 0.05*	+20	1.18 $\pm$ 0.07	-4
Anterior pretectal area	1.03 $\pm$ 0.04	1.24 $\pm$ 0.04*	+20	0.98 $\pm$ 0.04	-5
Reticular formation	0.70 $\pm$ 0.03	0.93 $\pm$ 0.04*	+33	0.66 $\pm$ 0.04	-6
Superior colliculus	1.12 $\pm$ 0.03	1.37 $\pm$ 0.05*	+22	1.21 $\pm$ 0.07	+8
Inferior colliculus	1.56 $\pm$ 0.04	1.95 $\pm$ 0.07*	+25	1.66 $\pm$ 0.08	+6
Central gray	0.81 $\pm$ 0.04	1.00 $\pm$ 0.05	+23	0.80 $\pm$ 0.05	-1
Dorsal tegmental nucleus	1.08 $\pm$ 0.04	1.30 $\pm$ 0.05*	+20	1.02 $\pm$ 0.06	-6
Metencephalon					
Pons	0.86 $\pm$ 0.03	0.92 $\pm$ 0.07	+7	0.76 $\pm$ 0.04	+12
Cerebellar gray (molecular)	0.80 $\pm$ 0.02	1.10 $\pm$ 0.04*	+38	0.86 $\pm$ 0.04	-8
Cerebellar gray	1.13 $\pm$ 0.04	1.43 $\pm$ 0.06*	+27	1.07 $\pm$ 0.06	-5
Dentate nucleus	1.12 $\pm$ 0.04	1.28 $\pm$ 0.08	+14	0.99 $\pm$ 0.09	-12
Vermis	0.97 $\pm$ 0.04	1.31 $\pm$ 0.04*	+35	0.98 $\pm$ 0.05	+1
Myelencephalon					
Vestibular nucleus	1.39 $\pm$ 0.04	1.66 $\pm$ 0.05*	+19	1.44 $\pm$ 0.07	+4
Cochlear nucleus	1.15 $\pm$ 0.04	1.43 $\pm$ 0.07*	+24	1.20 $\pm$ 0.07	+4
Superior olive	1.29 $\pm$ 0.05	1.56 $\pm$ 0.06*	+24	1.36 $\pm$ 0.06	+5
Inferior olive	1.00 $\pm$ 0.04	1.16 $\pm$ 0.05	+16	0.91 $\pm$ 0.07	-9
Spinal nerve trigeminal	0.84 $\pm$ 0.02	1.02 $\pm$ 0.04*	+21	0.76 $\pm$ 0.03	-10
Reticular formation	0.82 $\pm$ 0.02	0.97 $\pm$ 0.04*	+18	0.73 $\pm$ 0.04	-11
Whole brain	0.96 $\pm$ 0.02	1.20 $\pm$ 0.03*	+25	0.96 $\pm$ 0.04	+4

Fig. 1. Regional brain glucose use in normal and diabetic rats

Glucose use was measured in normal, 1-week-diabetic (○) and 4-week-diabetic (▲) rats as described in the Materials and methods section. Each point represents the mean value in one brain area in the diabetic group plotted against the corresponding value in the normal group. The line shown has a slope of 1. A straight line drawn through the 1-week values had a slope of 1.25, which was significantly greater than 1. The line drawn through the 4-week values was not different from the line of identity.

1975; Hawkins *et al.*, 1986). Because ketone-body concentrations in brain tissue remain unmeasurable, it is assumed that they are metabolized as rapidly as they enter, thus making a contribution to the total fuel use of the brain (Hawkins & Biebuyck, 1979). If the energy requirement of the brain is unchanged under these conditions, the consumption of glucose would be expected to decrease. At 4 weeks after streptozotocin injection, ketone bodies were estimated to contribute about 7–15% to the total energy requirement (assumed to be normal) (Hawkins *et al.*, 1986). Thus glucose use would be expected to be commensurately lower, unless total energy requirements were higher.

Results from some studies have led to the suggestion that glycolysis may be inhibited at phosphofructokinase by increased ketone-body oxidation. In streptozotocin-diabetic rats anaesthetized with pentobarbital, brain fructose 6-phosphate content was increased and fructose 1,6-bisphosphate content decreased, supporting this idea (Ruderman *et al.*, 1974). Furthermore, brain content of citrate, which may inhibit phosphofructokinase under these conditions (Passonneau & Lowry, 1964), was increased in this and another similar study of unanaesthetized rats (Blackshear & Alberti, 1974). It has also been suggested that pyruvate oxidation may be inhibited; however, Ruderman *et al.* (1974) found no increase in lactate efflux. In both these studies (Ruderman *et al.*, 1974; Blackshear & Alberti, 1974), a rather severe model of rapidly progressing ketoacidosis produced by 150 mg of streptozotocin/kg was used, which may produce different effects from the relatively more stable state caused by 65 mg/kg, as in our study. Also, the use of

anaesthesia in the study by Ruderman *et al.* (1974), which may conceivably affect diabetic rats differently from normal rats, may complicate the picture. However, in general, the idea that brain glucose use should decrease as ketone-body use increases remains attractive. For this reason, our findings were somewhat unexpected.

Before drawing conclusions, several factors which can affect the method of measurement of glucose use were considered. Transport of glucose into the brain may be decreased in rats with moderate diabetes. Between 1 and 25 days after streptozotocin injection, glucose transport was depressed by one-third, as measured by the brain-uptake-index method (McCall *et al.*, 1982). The maximal capacity was decreased by 45% (McCall *et al.*, 1982). In a similar study the maximal transport capacity was decreased by 29% at 3 weeks (Gjedde & Crone, 1981). However, the brain-uptake-index method of measuring glucose transport may give misleading results under these circumstances. Computer simulations which take into account both membranes of the capillary endothelial cell forming the blood/brain barrier predict that, when brain glucose content is substantially elevated, as it is during diabetes, the brain-uptake-index method may cause underestimation of the activity of the transport system (G. A. Oyler & R. A. Hawkins, unpublished work). This phenomenon is due, at least in part, to the fact that during passage of the brain-uptake-index injection through the capillaries, glucose moves from brain tissue to the capillary lumen, thereby diluting the radioactive glucose tracer. Thus the movement of the tracer into the brain is impeded by competition with non-radioactive glucose, and therefore uptake is retarded. This effect is greatest when brain glucose content is high, as it is in diabetes, and when low tracer concentrations are used. Another approach, which avoids this error, is to use steady-state analysis based on the relationship: $\text{influx} = \text{efflux} + \text{metabolism}$, as described by Gjedde & Christensen (1984). Thus, knowing the concentrations of plasma glucose and brain glucose, and the rate of glucose use, one can check whether the observed brain/plasma glucose ratio is compatible with a changed or an unchanged rate of glucose transport. With our data, such an analysis indicated that there was no alteration in the transport system, i.e. the brain glucose content in diabetic rats could be predicted correctly on the basis of normal transport kinetics. Therefore, because we could find no evidence for an effect of diabetes on glucose transport, it was assumed to be normal in the calculation of the results in our study. However, if a decrease in transport were present, it would lead to an underestimate in our values of glucose use for the diabetic rats, and therefore any correction would increase the difference between the normal and diabetic rats by making the values for the latter even higher.

Another transport system that is affected during ketonaemia is the monocarboxylic acid system, which transports the ketone bodies as well as lactate and pyruvate into the brain. Transport of 3-hydroxybutyrate and acetoacetate has been found to be increased during diabetes (Hawkins *et al.*, 1986); however, in a different study lactate transport was not affected (McCall *et al.*, 1982). An increased capacity of the monocarboxylic system might lead to loss of label as [^{14}C]lactate during the 8 min experimental period used for the measurement of glucose use in our study, and introduce an error. However, the measurement of arteriovenous differences

showed that the loss of label during this period was insignificant (no more than 2.7–3.4% of brain ^{14}C), and not different among the various groups.

Arteriovenous differences are technically difficult to measure in small animals; however, our results are in general agreement with those of Ruderman *et al.* (1974), showing no significantly greater loss of lactate from the brain during streptozotocin-diabetes. This is compatible with our finding that glucose use by brain tissue during diabetes is not lowered.

The difference observed in glucose use between the 1-week- and 4-week-diabetic rats in our study may reflect merely the greater availability of ketone bodies for brain energy consumption, because of the much greater elevation in plasma concentrations at 4 weeks (Table 1). In a study of the 3-week-streptozotocin-diabetic rat, glucose use was found to be decreased by 13% (Duckrow & Bryan, 1987). The reasons for the different findings in this study are not obvious, especially since the method used was similar to ours. One possibility is simply that the plasma ketone-body concentrations (which were not reported) were much greater in the diabetic Sprague-Dawley rats studied by Duckrow & Bryan (1987) than in the Long-Evans rats used in our study. We have noted previously that Long-Evans rats do not appear to become as ketotic during starvation or diabetes as other strains [e.g. compare Hawkins *et al.* (1971*b*) and Ruderman *et al.* (1974) with Hawkins *et al.* (1986)].

Although the increased brain use of ketone bodies during ketonaemia is expected to contribute to energy metabolism and to spare glucose if the total energy requirements are normal, the distribution of rates of use of these two fuels are not entirely superimposable. This has been demonstrated for normal rats (Hawkins & Biebuyck, 1979), and appears also to be the case in diabetic rats, as judged from the appearance of the autoradiographs and quantitative measurements (Hawkins *et al.*, 1986). This means that ketone-body consumption cannot be expected to supplement glucose use in a straightforward manner on a regional basis. Nevertheless, all parts of brain do derive energy from ketone bodies. In conclusion, brain energy consumption appears to be higher than normal during diabetes. At 1 week, when ketone bodies were increased, but not as high as at 4 weeks, this was shown by an increase in glucose use throughout the brain. At 4 weeks glucose use was normal, but additional energy was available from ketone bodies.

This study was partially supported by N.I.H. grant NS16737. We thank Miss Jean Heisey for secretarial assistance.

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