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Use of nonradioactive 2-deoxyglucose to study compartmentation of brain glucose metabolism and rapid regional changes in rate

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ABSTRACT **A method is presented for measuring rapid changes in the rate of glucose phosphorylation in mouse brain with nonradioactive 2-deoxyglucose (DG). After times as short as 1 min after DG injection, the mouse is frozen rapidly, and selected brain regions are analyzed enzymatically for DG, 2-deoxyglucose 6-phosphate (DG6P), and glucose. The rate of glucose phosphorylation can be directly calculated from the rate of change in DG6P, the average levels of DG and glucose, and a constant derived from direct comparison of the rate of changes in glucose and DG6P after decapitation. Experiments with large brain samples provided evidence for a 2% per min loss of DG6P and at least two compartments differing in their rates of glucose metabolism, one rapidly entered by DG with glucose phosphorylation almost double that of average brain and another more slowly entered with a much lower phosphorylation rate. The method is illustrated by changes in phosphorylation within 2 min after injection of a convulsant or an anesthetic and over a 48-min time course with and without anesthesia. The sensitivity of the analytical methods can be amplified as much as desired by enzymatic cycling. Consequently, the method is applicable to very small brain samples. Examples are given for regions with volumes of 5×10^{-4} μl, but studies with samples as small as single large cell bodies are feasible.**

The nearly exclusive dependence of the brain on glucose for energy and the close relationship between energy metabolism and neuronal activity have been exploited in the classic 2-[^{14}C]deoxyglucose ([^{14}C]DG) method of Sokoloff *et al.* (1) to map patterns of neural activity in a wide variety of physiological and drug-induced states. The number of studies that have used this method shows the great demand for information about regional brain activity. Nevertheless, as useful as the method has proven to be, it has one basic limitation, i.e., the necessary 30- to 45-min lag period between DG injection and brain fixation. The lag is needed to allow free DG to largely dissipate, since it is the 2-deoxyglucose 6-phosphate (DG6P) accumulation that is the index of glucose phosphorylation and hence of its metabolism. Many investigators are interested in brain events that take place in a much shorter time frame.

This paper presents a DG procedure with temporal resolution of a minute or less. The method depends upon direct measurement of DG, DG6P, and glucose without physical separation (2). The sensitivity is sufficient to assay samples as small as, or smaller than, the areas resolved in the usual radioautographs. Although a larger than tracer dose of DG is required, this is kept low enough not to significantly distort glucose metabolism. The assessment of glucose phosphorylation from directly observed levels of the primary metabolites concerned (DG, DG6P, and glucose) avoids many of the uncertainties that exist when these metabolites are calculated from plasma DG levels. In working out the method, some features of brain glucose metabolism have become evident that probably must be considered in any study of this kind, whether the time scale is short or long.

MATERIALS AND METHODS

CF1 mice were used, usually females (Harlan). They were handled twice daily for a week to render them less excitable. DG was injected into a lateral tail vein in a volume of 10 ml/kg (body weight). Injection time was 2–4 sec. Animals were usually killed by immersion in liquid N_2. The brain was removed at −20°C. Large samples were removed and weighed at this temperature or sections were cut on a microtome at −20°C and freeze-dried at −35°C. Dissection and weighing of small samples from these sections was performed at 20–23°C. For procedural details, see ref. 3. Plasma for DG assays was obtained from blood collected after decapitation.

Analytical Procedures. Measurement of DG, DG6P, and glucose depends on the facts (*i*) that glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49) reacts with DG6P, but at a 2000-fold slower rate than with glucose 6-phosphate and (*ii*) that hexokinase reacts rapidly with both DG and glucose. Therefore, NADPH produced with a low level of glucose-6-phosphate dehydrogenase measures glucose 6-phosphate, whereas that produced with a much higher level measures DG6P. Glucose and DG are similarly distinguished after phosphorylation with hexokinase (2). Experience with various kinds of brain samples has turned up problems that had to be solved for optimal results (4).

RESULTS

The basic equation for assessment of glucose phosphorylation is

$$\Delta g = \Delta p(g/d)C, \qquad [1]$$

where C is a constant assumed to be the same for all regions of the brain, p is DG6P, g is glucose, and d is DG. g and d represent their average concentrations over the time interval concerned.

Calculation of *C* for Eq. 1. C is an estimate of the ratio between the velocities of hexokinase with DG and glucose. We concluded that the unambiguous way to determine C would be to compare the rate of glycolysis with the rate of accumulation of DG6P after decapitation. Anesthetized mice were injected with DG, decapitated 2 min later, and the heads were frozen after another 15–115 sec. The 15-sec delay before the first freezing time was to allow consumption of the oxygen present. Anesthesia slowed events enough for accu-

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Abbreviations: DG, 2-deoxyglucose; DG6P, 2-deoxyglucose 6-phosphate; GPR, glucose phosphorylation rate; PTZ, pentylenetetrazole.

rate timing. Glucose, DG, DG6P, lactate, and glycogen were measured in 50-mg samples from the dorsal surface of the cerebral hemispheres (Table 1). ATP and phospho-creatine were also measured (Table 1) to permit assessment of metabolic rate (Table 2).

Since the brain had been converted to a closed system by decapitation and oxygen was excluded, most of the glucose metabolized would end up as lactate, and DG would be converted in parallel to DG6P. Consequently, from Eq. **1**: $C = (\Delta g/\Delta p)(d/g)$. Lactate increase (divided by 2) was used to confirm the rate of glucose phosphorylation. Because the percentage changes were much greater in the case of lactate, they were far less susceptible to animal variation (Table 1). As judged from an earlier study in which the changes in glycerol phosphate and all the intermediates between glucose and lactate were measured, lactate increase may underestimate glucose decrease by ≈10% (5). Although the pivotal enzyme is hexokinase, its kinetic constants do not enter into the calculation. As predicted, the constant calculated from the lactate increases are more consistent than those from glucose decreases, and the average value is ≈10% lower (Table 2). A consensus figure for C is 2.8.

The metabolic rate, calculated as indicated in the legend of Table 2, is equivalent to ≈10 mmol of $\approx P \cdot kg^{-1} \cdot min^{-1}$. This is 40% of the value calculated many years ago for unanesthetized mice (5).

Time Curves for DG in Blood Plasma and for DG and DG6P in Whole Brain. To provide background for studies of rapid changes in glucose metabolism and to establish linkage with radioautographic tracer methods, determinations were made over a long time course of DG in plasma and of DG and DG6P in large samples of the dorsal cerebrum (designated "brain"). The period chosen, 48 min, covers the full period of the usual Sokoloff method (1).

In controls, plasma DG level dropped almost 50% between 1 and 6 min and then declined more slowly reaching 5% by 48 min (Fig. 1). In deeply anesthetized animals, the initial fall of plasma DG was similar, but decelerated more rapidly, leaving 25% of the 1-min level at 24 min and 8% at 48 min (Fig. 1).

Much bigger differences in brain DG were observed between control and anesthetized mice. Control levels were maximal at ≈1 min (Fig. 1) and varied remarkably little from 0.25 to 3 min (Fig. 2 *Inset*). The level then fell to half by 12 min and was still 16% of the peak at 48 min (Fig. 1). Under anesthesia, DG continued to climb for the first 6 min, reaching a peak 50% higher than in control brain (Figs. 1 and 3). The subsequent decline was less than in controls, so that by 48 min the level was 31% of the peak and 3 times that of 48-min controls in absolute terms. Although plasma DG concentrations were at all times higher in anesthetized than in control mice, the differences are too small, particularly at early times (3–12 min) to explain the much greater differences in brain DG.

DG6P in control brain increased almost linearly for 3 min, reached a peak at 12 min, and then fell 35% over the next 48 min (Fig. 2). In anesthetized mice, in spite of the higher DG levels, the DG6P rose much more slowly than in controls, reaching the highest level at 24 min with a 6% (nonsignificant) fall by 48 min (Fig. 3). This peak DG6P level was only 31% as high as the peak in controls. (Note the much larger vertical scale in Fig. 3 than in Fig. 2). The fraction that DG6P contributed to the sum of DG plus DG6P was 50%, 80%, and 89% in controls at 3 min, 12 min, and 48 min, respectively. In anesthetized animals at these same times, the DG6P fractions were only 9%, 28%, and 57%, respectively, of the totals. Thus, under deep anesthesia, the DG level after 48 min was still almost as high as that of DG6P, an observation of major importance for interpretation of radioautographic studies.

Table 2. Evaluation of the constant C, relating glucose phosphorylation to DG phosphorylation

Time, sec	C from Δg	C from Δlac	Metabolic rate, mmol ~P per kg per min
15–40	3.70	2.60	10.5
40–65	2.88	2.75	10.7
65–115	2.35	2.50	10.0
Average	2.98 ± 0.39	2.62 ± 0.07	10.4 ± 0.2

These calculations of C are based on the data in Table 1 as follows: $C = (\Delta g/\Delta p)(d/g)$ or $C = (\Delta \text{lac}/2p)(d/g)$, where d and g represent the average concentrations of DG and g, respectively, over the interval concerned. The metabolic rates are calculated from the Table 1 data on the assumption that the molar ratio between ~P generated and the change in each metabolite is 1 for phosphocreatine and for lactate from glucose, 1.5 for lactate from glycogen, and 1.4 for ATP [an approximation for the expected ratio of ADP to AMP generated (5)].

Brain glucose levels (data not shown) were measured in all samples of the experiments of Figs. 1–3. This permitted calculation of the apparent glucose phosphorylation rates (GPRs) for various time intervals from Eq. **1** if loss of DG6P could be ignored. Glucose averaged 1.87 mmol/kg of H_2O in the control experiment with no time trend. The calculated control GPRs from 0.25 to 3 min, 3 to 6 min, and 6 to 12 min were 1.53, 1.35, and 0.58 mmol per kg of H_2O per min, respectively, and then became negative as DG6P levels actually decreased. If the average GPR for the first 6 min had persisted for the full 48 min and DG6P loss had not occurred, the expected DG6P change would have followed the "Calc A" curve of Fig. 2. By 48 min, observed was only 27% of expected on these assumptions.

At least two possibilities could explain the huge discrepancy. (*i*) DG6P might be lost at a rather high rate. To explain the discrepancy on this basis alone, DG6P would have to be lost at a first-order rate of ≈6% per min (Fig. 2, Calc B curve). (*ii*) At least two compartments are involved, one with a relatively high GPR and rate of DG entry and another where both glucose metabolism and DG entry are substantially slower. For example, the observed data could be explained on this basis if DG6P was lost at a rate of 2% per min and the GPR in the slower compartment was only 12% of that in the fast.

Table 1. Changes in metabolites of glucose and DG in brain after decapitation

Time, sec	Compound, mmol/kg						
	DG	DG6P	Glucose	Lactate	Glycogen*	ATP	P-creatine
15	0.752 ± 0.017	0.053 ± 0.007	7.27 ± 0.47	2.62 ± 0.10	1.81 ± 0.10	1.89 ± 0.11	1.90 ± 0.06
40	0.679 ± 0.018	0.124 ± 0.015	5.01 ± 0.39	5.85 ± 0.20	1.79 ± 0.02	1.77 ± 0.12	0.95 ± 0.05
65	0.583 ± 0.024	0.213 ± 0.017	3.32 ± 0.34	9.23 ± 0.14	1.76 ± 0.05	1.40 ± 0.08	0.44 ± 0.02
115	0.372 ± 0.035	0.433 ± 0.035	1.05 ± 0.18	15.27 ± 0.94	1.16 ± 0.04	0.38 ± 0.06	0.10 ± 0.01

Mice were anesthetized for 30 min with chloral hydrate, then injected with DG (1 mmol/kg) and decapitated 2 min later, and the heads were frozen in liquid N_2 after the subsequent time intervals shown. Samples (50 mg) were taken for analysis from the dorsal surface of the frozen brain. P-creatine, phosphocreatine.
*Glucose equivalents.

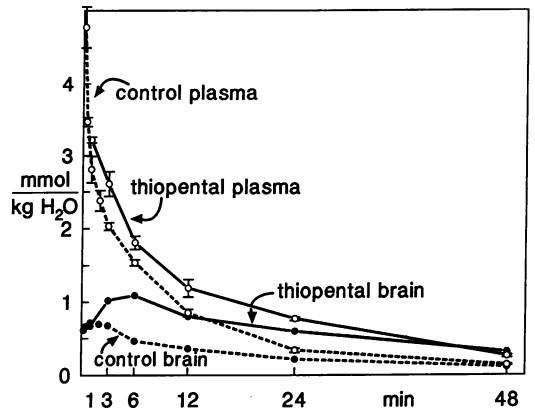

FIG. 1. DG levels in plasma and brain of control and anesthetized (thiopental) mice. Control animals received 1 mmol of DG per kg (i.v.). Anesthetized animals received 1.4 mmol of DG per kg; to compensate for the larger dose, all data have been divided by 1.4. Thiopental (125 mg/kg, i.p.) was given 10 min before the DG. Each point represents the average for three mice. SE values are shown for the plasma levels; SE values for brain levels are given in Figs. 2 and 3. A 50-mg sample from the dorsal cortex was taken for analysis. The data are calculated on the basis of H_2O, assuming H_2O content of 80% in brain and 94% in plasma. A deduction was also made from the brain DG equal to 2.5% of the plasma DG level (according to unpublished data based on the distribution volume of G6PDH from *Leuconostoc mesenteroides*).

Although some loss of DG6P undoubtedly occurs, the magnitude in the rat has usually been estimated to be much less than 6% per min, which makes the second explanation of the present results more plausible than the first. In support of this, it was found that if thiopental was injected 20 min after DG to greatly diminish DG phosphorylation (by lowering the metabolic rate and increasing the glucose/DG ratio), the rate of fall in DG6P was reduced to 1.8% per min.

In the anesthetized mice, average brain glucose was 4.88 mmol/kg of H_2O, 2.6 times that of control brain. As noted, this increase is shared to some extent by DG (Fig. 1). (The effect of anesthetics to increase brain glucose is an old observation (5, 6), often overlooked.) Application of Eq. 1 to the initial linear rate of DG6P increase gave a GPR of 0.458 mmol per kg of H_2O per min, indicating a 71% inhibition by the anesthetic. As with control mice, DG6P concentrations after 6 min were much lower than calculated from the initial rate, although the discrepancy was not as great (Fig. 3, Calc A curve). It could be explained by loss of DG6P alone if the rate of loss was 3.5% per min (Fig. 3, Calc B curve), or a combination of DG6P loss of 2% per min and a GPR in the

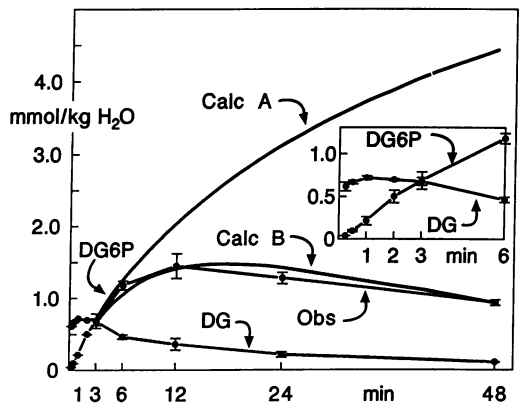

FIG. 2. DG6P levels in the brains of the control mice of Fig. 1. DG levels are repeated from Fig. 1. SE values are indicated. "Calc A" and "Calc B" are explained in the text.

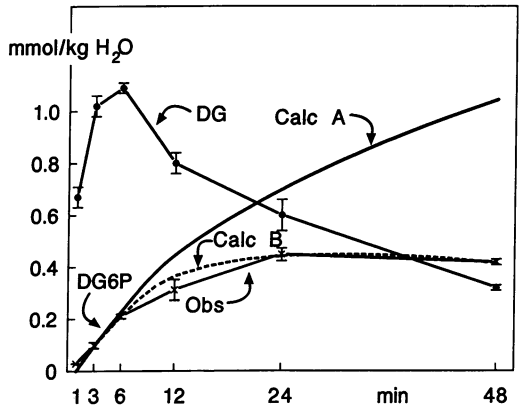

FIG. 3. DG6P and DG levels in the brains of the anesthetized mice of Fig. 1. SE values are indicated. Calc A and Calc B are explained in the text. Note the larger vertical scale than in Fig. 2.

hypothetical slow compartment that is 61% of that in the fast compartment. If this interpretation of the data should be correct, it would indicate that glucose metabolism is less affected by the anesthetic in the slow compartment than in the fast.

The question comes up whether these results, with much larger than the usual tracer amounts of DG, have relevance for radioautographic studies. Huang and Veech (7) made a study similar to that of Fig. 1 with unanesthetized rats except for the use of a tracer dose of [^{14}C]-DG. Brain metabolism was stopped by "freeze-blowing" (blown into a freezing chamber) and [^{14}C]DG and [^{14}C]DG6P were separated chromatographically and measured. The results, though less consistent, were much like those of Fig. 1 and could be explained in the same way (as the authors also calculated) by persistence of the apparent initial GPR plus a rapid rate of DG6P loss (5% per min). In another tracer study (8), the results also indicated a much faster accumulation of DG6P relative to DG during the first 5 min than in the subsequent 45 min. A cumulative loss of DG6P at a rate of 4.5% per min would account for the final DG6P concentration, as would a lesser loss from compartments differing in GPR.

Anesthetic Dose-Response Results. Chloral hydrate was chosen for a dose–response study (Fig. 4) since there is no sign that it causes stimulation with subanesthetic doses. The dose was increased in five equal steps. The lowest dose produced ataxia with no decrease in spontaneous activity or loss of righting reflex; the two highest doses caused complete anesthesia and unresponsiveness. Ten minutes after giving the anesthetic i.p., DG was injected. The mice were frozen 2 min later. Brain DG and glucose rose progressively by totals of 50% and 300%, respectively (Fig. 4 *Middle*). DG6P leveled off at about 15% of control. The changes of $[p/(g/d)]$ (Fig. 4 *Right*) indicate decreases in GPR from 21% to 72%—i.e., about the same maximal effect as with thiopental above.

Regional Metabolic Studies. This section provides examples of the use of the proposed method with sample sizes down to 0.1 μg (dry weight). To simplify the procedure, the data are limited to DG6P and DG. A change in the DG6P/DG ratio is at least as sensitive an indicator of a change in glucose phosphorylation as a change in calculated GPR.

In an earlier report (2), the effects of the convulsant pentylenetetrazole (PTZ) on whole-brain GPR were assessed by the proposed procedure (2). Calculated GPR in the interval from 0.5 to 1.5 min after PTZ injection was nearly 3 times control. To assess the effect of PTZ in major cerebral subdivisions, 20-μm coronal sections at the level of the caudal end of the striatum were made from two mice frozen 3 min after a DG injection. One had received PTZ 2 min before freezing. A section from one hemisphere was divided

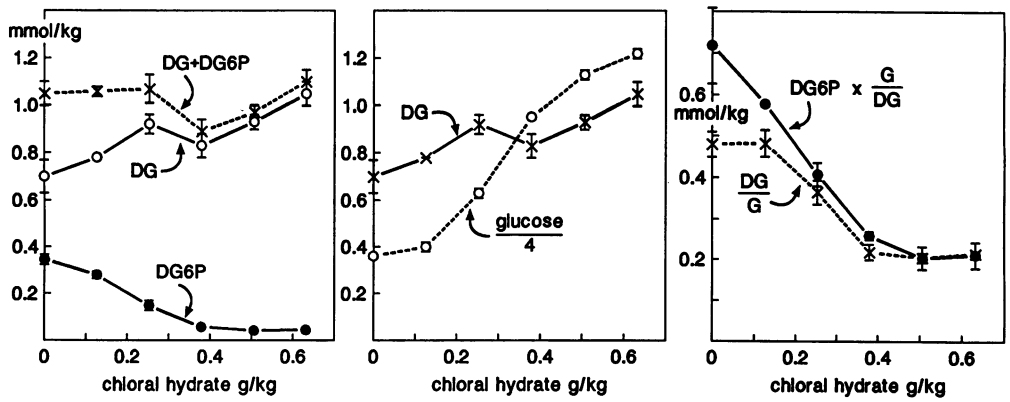

FIG. 4. Chloral hydrate dose–response curves for DG, DG6P, and glucose in 50-mg samples from the dorsal cortex. DG was given (1 mmol/kg, i.v.) 10 min after giving chloral hydrate (i.p.) at the doses indicated. The mice were killed 2 min later by immersion in liquid N_2. Each point represents data for two mice. The error bars represent the positions of the two values.

for assay into five areas, each with a dry weight of ≈10 μg (Fig. 5). In the two cortical regions PTZ caused a 50–130% increase in DG6P and an equivalent fall in DG, whereas in the three subcortical zones there were no significant changes. From adjoining sections, the cortical region most severely affected by PTZ was subdivided into six portions, ≈2 μg in weight (indicated by dotted lines in Fig. 5). All six showed major effects of PTZ without substantial differences among them (data not shown). However, in all of these samples, DG had dropped so low as to suggest that in some cells DG may have been completely depleted and, therefore, no longer a measure of glucose metabolism. In spite of the major decreases in DG, the sum of DG plus DG6P did not change—i.e., there was no significant compensatory increase in the influx of DG from the bloodstream. Samples weighing ≈5 μg from six fiber tracts of these same two brains were also assayed (Fig. 6). The sum of DG plus DG6P on the basis of H_2O content would be roughly the same as for the five areas of Fig. 5. Therefore, these tracts would not qualify as representative of compartments more slowly penetrated by DG than average brain. A PTZ effect was seen in the corpus callosum, with a lesser effect in the anterior commissure. This indicates that the seizure affected neurons that give rise to the interhemispheric fibers. The lack of effect on fornix and fimbria is in keeping with the absence of change in the hippocampal region of Fig. 5. The control ratios of DG6P to DG were much lower in corpus callosum, fornix, and fimbria than the average for cerebrum. This presumably indicates a lower GPR, but concurrent glucose levels are needed to make sure.

Fig. 7 illustrates the effect of kainic acid on four regions of area CA1 of the hippocampus and layer V of the adjacent cortex. The mice were given simultaneously both DG and kainic acid (20 mg/kg) and frozen 10 min later. The samples for assay weighed 0.1–0.15 μg (dry weight). The smallest change in the DG6P/DG ratio is seen in stratum oriens and the cortical sample. The results are provisional since each region is represented by only one control and one experimental sample, but they demonstrate the practicality of applying the proposed method to very small areas.

DISCUSSION

The results clearly suggest that the proposed method can assess changes in the rate of brain glucose phosphorylation on a time scale of 1–3 min, and on a regional basis with samples as small as 0.1 μg (dry weight). However, what may be more important is the information this method can give about basic brain metabolism. So far, this includes specific data about compartmentation of glucose metabolism and the differential effects of anesthesia on glucose and DG.

The assessment of glucose phosphorylation from the results of a dose of DG, whether radioactive or not, must

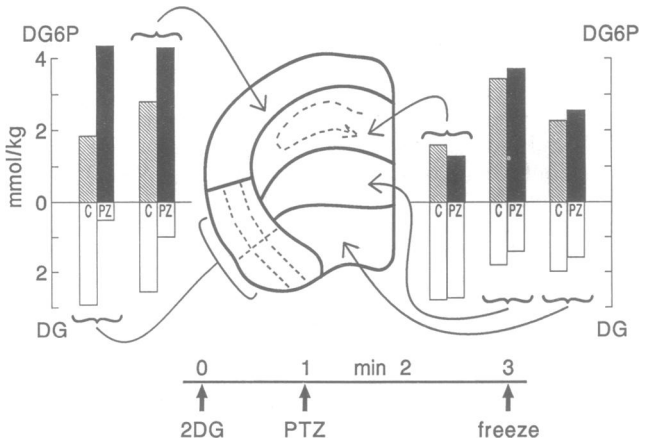

FIG. 5. Effect of PTZ (or PZ) on DG6P and DG in five regions of a coronal section of one hemisphere, at the level of the caudal end of the striatum. Each portion weighed ≈10 μg (dry weight). C, similar samples from a section of control brain. One of the five regions from an adjacent section was subdivided into six parts for assay, as shown by the dotted lines (see text). The PTZ dose was 100 mg/kg (i.p.); the DG dose was 1 mol/kg (i.v.).

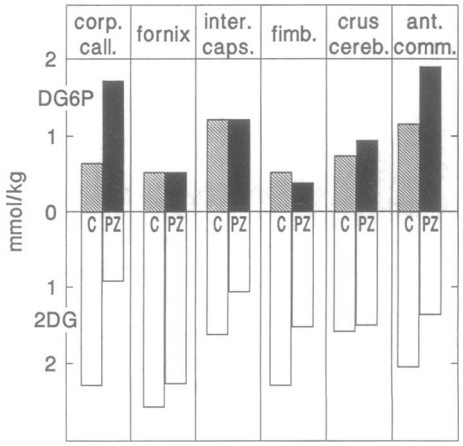

FIG. 6. Effect of PTZ (PZ) on six tracts dissected from freeze-dried sections of the same brains represented in Fig. 5. C, tissue from the control brain; corp call, corpus callosum; inter caps, internal capsule; fimb, fimbria; crus cereb, crus cerebri; ant comm, anterior commissure.

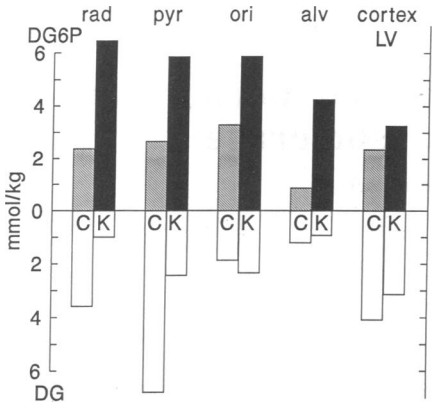

FIG. 7. Effect of kainic acid (K) on DG6P and DG of four regions of area CA1 of the caudal hippocampus and layer V (LV) of the adjacent cerebral cortex. C, control brain. Sample dry weights were 0.1–0.15 μg. Kainic acid (20 mg/kg) and DG (1 mmol/kg) were given together, i.v., 10 min before the mice were frozen. rad, radiata; pyr, pyramidalis; ori, oriens; and alv, alveus.

depend on values for DG, DG6P, and glucose observed, calculated, or assumed. This nonradioactive DG method provides values of these parameters directly. The radioautographic method provides them indirectly by calculation from a combination of a time course of plasma DG levels, the final regional radioactivity, and a "lumped" constant. It is also usually assumed that glucose levels are constant throughout the brain. The present results give examples of changes in these key parameters, induced by experimental manipulation, that the Sokoloff type of calculation could not be expected to take into account. Thus, anesthesia increased the amount of DG remaining 48 min after injection from 11% in controls to 43%; this would completely distort calculations made on the basis of final radioautographic results. Similarly, the direct assay data showed that deep anesthesia increases brain levels of glucose and DG, but the DG increase is not as great as that of glucose, which again would throw off the calculations. As mentioned in the text, there have been a few special experiments in which [^{14}C]DG and [^{14}C]DG6P were measured separately, but since this required quick freezing by freeze blowing, followed by column separation, it was only applicable to whole brain, and the reported results have been quite erratic, as well as time consuming.

Nelson *et al.* (9) have pointed out that brain compartments with higher than average metabolic rates are likely to have higher rates of blood flow and DG uptake and will, therefore, generate higher than average DG6P at early times after DG injection. They further concluded that eventually the slower compartments would become better represented and contribute DG6P in proportion to their glucose metabolism and relative mass. Our results agree with this, but they indicate that the rate of DG6P formation after the first 10 or 15 min is dominated by the glucose phosphorylation rates in the slowest compartments. Regional studies with this method covering early and late time intervals should help clarify this issue.

Can Nontracer DG Loads Provide Valid Information About Normal Glucose Metabolism? We believe the data presented demonstrate that if the DG load is not much in excess of 1 mmol/kg (body weight), it will not cause a major disturbance in brain metabolism. During the time of peak brain DG concentration, brain hexose phosphorylation would be divided 12 or 15 to 1 between glucose and DG—i.e., glucose phosphorylation would be inhibited <8%. After longer times the inhibition would be even less. Even this modest inhibition would be counteracted by normal enzyme control mechanisms.

Tissue Fixation Requirements. As with any invasive study of metabolically labile brain constituents, rapid fixation is required to preserve *in vivo* levels. For example, in the absence of anesthesia, after 5 sec of complete ischemia (decapitation), levels in whole brain of DG as well as glucose are cut in half with a corresponding increase in DG6P (data not shown). Without anesthesia, the mouse is about the largest animal in which the head can be frozen fast enough to approximate *in vivo* levels. Even in the mouse, there will be some ischemic changes before deeper brain regions are frozen. Up to a point, this does not invalidate studies of deep-lying regions by the proposed method, since control and experimental samples from these regions will have been exposed to the same freezing delay. It is possible that microwave fixation offers a solution to this fixation problem, even for larger animals (10).

Other Procedures for Assessing Rapid Changes in Brain Glucose Metabolism. Cunningham and Cremer (10) have developed a DG procedure for assessing glucose phosphorylation over intervals as short as 2 min with brain sample sizes on the order of 1–5 mg. [^{3}H]DG and [^{14}C]DG are injected separately at 2-min intervals and the animal (rat) is killed by microwave irradiation 5 min after the first injection. Extracts of brain regions are separated on ion-exchange columns into DG and DG6P fractions, and ^{3}H and ^{14}C are measured in these fractions. This permits direct measurement of the increment in DG6P over the 2-min time span between the two injections. The need for large samples and physical separation of the DG6P from DG are drawbacks.

Hawkins *et al.* (11) developed a radioautographic method that depends on the use of glucose itself labeled with ^{14}C. The experiment is terminated before a significant amount of [^{14}C]CO_2 leaves the brain (5 or 10 min) at which time the ^{14}C is divided between glucose and its metabolic products. Correction is made for the ^{14}C in brain glucose, on the assumption that glucose is constant throughout the brain and that its specific activity at the termination of the experiment can be calculated from the time course of plasma [^{14}C]glucose. This presents a problem, since a change in the local rate of glucose metabolism should in itself affect the local glucose concentration. Anesthesia, for example, as emphasized here, increases markedly the ratio of brain to plasma glucose, presumably because it decreases the rate at which brain glucose is removed by metabolism without a corresponding decrease in its influx from the blood (6).

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