Extracellular Brain Glucose Levels Reflect Local Neuronal Activity: A Microdialysis Study in Awake, Freely Moving Rats

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Abstract: The relationship between brain extracellular glucose levels and neuronal activity was evaluated using microdialysis in awake, freely moving rats. The sodium channel blocker tetrodotoxin and the depolarizing agent veratridine were administered through the dialysis probe to provoke local changes in neuronal activity. The extracellular glucose content was significantly increased in the presence of tetrodotoxin and decreased sharply following veratridine application. The systemic injection of a general anaesthetic, chloral hydrate, led to a large and prolonged increase in extracellular glucose levels. The brain extracellular glucose concentration was estimated by comparing dialysate glucose efflux over a range of inlet glucose concentrations. A mean value of 0.47 mM was obtained in five animals. The results are discussed in terms of the coupling between brain glucose supply and metabolism. The changes observed in extracellular glucose levels under various conditions suggest that supply and utilization may be less tightly linked in the awake rat than has previously been postulated. **Key Words:** Glucose—Tetrodotoxin—Veratridine—Anaesthesia—Microdialysis—Rat brain. **Fellows L. K. et al.** Extracellular brain glucose levels reflect local neuronal activity: A microdialysis study in awake, freely moving rats. *J. Neurochem.* **59,** 2141–2147 (1992).

The brain is singularly dependent on glucose as its main source of energy (Siesjö, 1978). Although astrocytes do contain small amounts of glycogen (Swanson et al., 1990), the brain relies overwhelmingly on glucose supplied through the blood (Lund-Andersen, 1979).

The extracellular glucose concentration represents a balance between supply from the blood and intracellular utilization. Glucose is taken up into the brain by facilitated transport across the blood–brain barrier (BBB). Under normal conditions, supply is in excess of metabolic requirements (Pardridge, 1983).

The rates of glucose uptake and utilization are, however, not fixed. Hexokinase is an important regulatory enzyme in the glycolytic pathway, and its activity is sensitive to changes in the cellular energy balance (Siesjö, 1978). There is evidence that both local glucose utilization (Sokoloff et al., 1977) and the rate of glucose transport (Braun et al., 1985) show regional variation and can change in a given region under different conditions.

Previous studies demonstrating the close coupling between glucose phosphorylation and glucose transport into the brain (Cremer et al., 1983; Hawkins, 1986) suggest that there should be little, if any, change in brain glucose concentration with changes in neuronal activity, except when there is a substantial decrease in plasma glucose concentration (in rats, below 2–2.5 mM) (Robinson and Rapaport, 1986; Pelligrino et al., 1990). Increases in the metabolic rate might challenge the glucose supply (Siesjö, 1978), but one model of brain glucose transport predicts that increases in the rate of glucose phosphorylation of 200–300% would still be adequately supplied from the blood (Robinson and Rapaport, 1986). The present study was designed to test this hypothesis.

Most previous studies have depended on ex vivo measurements. Monitoring glucose levels in the extracellular fluid (ECF) with microdialysis provides a powerful method for studying brain metabolism in awake, freely moving animals. We have examined the links between drug-induced changes in local neuronal

Abbreviations used: BBB, blood–brain barrier; ECF, extracellular fluid; GABA, γ-aminobutyric acid; TTX, tetrodotoxin.

activity and extracellular glucose levels, as well as the effects of general anaesthesia. The changes in extracellular glucose content observed under these conditions are discussed with respect to the local coupling between glucose transport and utilization.

EXPERIMENTAL PROCEDURES

Materials

Glucose oxidase and horseradish peroxidase were purchased from Boehringer-Mannheim. The silica beads used as the enzyme support substrate were from Merck. Tetrodotoxin (TTX), veratridine, and ferrocene monocarboxylic acid were obtained from Sigma Chemical Co. Chloral hydrate was from Hopkins and Williams. Kathon CG was from Rohm and Haas.

Glucose assay

The dialysate was analysed for glucose using a flow injection enzyme-based assay. The assay is described in detail elsewhere (Boutelle et al., 1992). In brief, glucose oxidase (EC 1.1.3.4) and horseradish peroxidase (EC 1.11.1.7) were immobilized on 10-μm spherical silica beads. The beads were highly activated with tresyl chloride and subsequently reacted with the appropriate enzyme. Approximately 60% of the sites available for enzyme binding were filled using this procedure.

The beads were slurry-packed into a 2- × 20-mm column (Anachem). A buffer solution, composed of 50 mM Na$_2$HPO$_4$, 1 mM EDTA, and 0.5 mM ferrocene monocarboxylic acid and adjusted to pH 7.0, with 0.05% Kathon CG added to inhibit bacterial growth, was pumped through the bed at 0.5 ml/min using an HPLC pump.

Dialysate was injected into the packed bed in 20-μl volumes. Glucose present in the sample was oxidized to give gluconolactone and H$_2$O$_2$. A second enzyme reaction was introduced at this stage, to optimize the electrochemical detection (Frew et al., 1986). The peroxide was oxidized to water by horseradish peroxidase, with the electrons transferred to the mediator compound ferrocene, which was present in the buffer. The ferricinium species produced was detected by reduction at a glassy carbon electrode, held at 0.0 V versus Ag$^+$/AgCl and located downstream of the enzyme bed. This series of reactions is summarized in Fig. 1.

The use of ferrocene as a mediator in this system has two advantages. First, it ensures the specificity of the assay, as the electrode potential can be held below that at which electroactive species in the dialysate would be oxidized. Second, the assay sensitivity is enhanced by avoiding the inefficient electrochemical detection of H$_2$O$_2$. The assay is linear over the physiological range of brain glucose concentration and has a detection limit of ~5 μM.

Probe construction

The microdialysis probes were of concentric design, constructed by inserting a plastic-coated silica tube (VS170/110; Scientific Glass Engineering) into a polyacrylonitrile dialysis fibre (o.d., 320 μm; Hospal, France). The fibre (total length, 5–6 mm) was glued into a stainless steel cannula, leaving an active length of 4 mm, and the tip was sealed with epoxy. A second silica tube inserted into the cannula served as the outlet.

Surgery

Male Sprague–Dawley rats (weighing 200–300 g) were anaesthetized with chloral hydrate (500 mg/kg i.p.) and placed in a stereotaxic frame. Body temperature was maintained at 37°C with a heating pad (Sandown Scientific, U.K.). The microdialysis probe was implanted into the right striatum (from bregma, A/P 1.0 mm, M/L 2.5 mm; from dura, −8.5 mm) and secured with skull screws and dental acrylate. The animals were then allowed to recover for 24 h. The health of the animals was assessed following recovery in accordance with published guidelines (Morton and Griffiths, 1985), and all procedures were specifically licensed under the Animals (Scientific Procedures) Act, 1986.

Experimental conditions

Following surgery, the rats were housed in large plastic bowls (Johnson's Garden Centre, Oxford, U.K.), with free access to food and water. Experiments were carried out with the animal in its home bowl. On experimental days, the probes were connected to a microinfusion pump (CMA Microdialysis) through a liquid swivel, allowing the animals free movement. The probes were perfused with an artificial CSF (composed of 147 mM NaCl, 4.0 mM KCl, 1.2 mM CaCl$_2$, and 1.0 mM MgCl$_2$), at 2 μl/min. Following a 30-min equilibration period, samples were collected every 10 min and analysed for glucose as described above.

In vitro recovery and estimation of extracellular concentration

The recovery of glucose with this probe design was evaluated in an unstirred 5 mM glucose solution. At 37°C, the in vitro recovery was 22%.

Values for in vivo recovery and the actual concentration of extracellular glucose were determined with the variation-of-concentration method (Lönnroth et al., 1987). The concentration of glucose in the perfusate being introduced into the probe was varied between 0 and 2.4 mM, and the net influx or efflux of glucose was measured in the dialysate at 2.5- or 5-min intervals. To minimize any initial perturbation of the probe environment, perfusion was begun with artificial CSF containing 0.8 mM glucose (an estimate of the extracellular concentration based on the in vitro recovery value), and the glucose concentration was then varied above and below this level. The actual concentration of glucose in the ECF was determined through regression analysis, by cal-
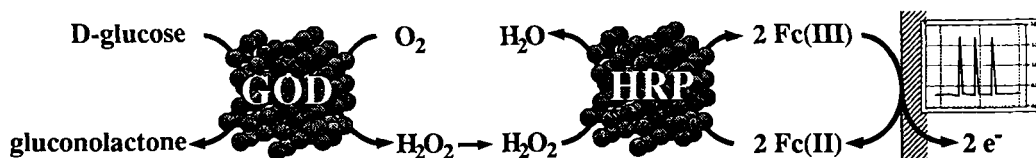

FIG. 1. Enzyme packed-bed flow injection assay for glucose. GOD, glucose oxidase; HRP, horseradish peroxidase; Fc, ferrocene.

culating the perfusate concentration where no net influx or efflux would occur.

Drugs

TTX and veratridine were dissolved in artificial CSF and applied locally through the probe, at concentrations of 1 and 50 μM, respectively. Neither drug interfered with glucose detection at these concentrations. Chloral hydrate was administered at a dose of 500 mg/kg i.p. as a 10% (wt/wt) solution in water. Anaesthetized animals were maintained at 37°C by means of a heating pad, as described for the surgery, and the depth of anaesthesia was monitored by periodic testing of the hind limb withdrawal reflex.

RESULTS

Basal glucose levels diminish on successive days

The basal concentration of glucose detected in the dialysate, while remaining constant over 3–4 h of continuous perfusion (data not shown), dropped significantly on succeeding days (Fig. 2). Thus, 72 h after implantation, the dialysate glucose concentration was reduced to 25% of the levels observed at 24 h. On this basis, all subsequent experiments were performed 24 h following probe implantation.

Determination of the basal extracellular glucose concentration

The actual concentration of glucose in the brain ECF was determined in five animals (Fig. 3), by varying the perfusate glucose concentration as described in Experimental Procedures.

To avoid disturbing the probe environment, perfusion was begun at a glucose concentration near the expected ECF level (0.8 mM). At all glucose concentrations, the perfusate equilibrated rapidly with the extracellular pool; steady-state conditions were achieved within 10 min of changing the perfusion concentration. At a given perfusate glucose level, the
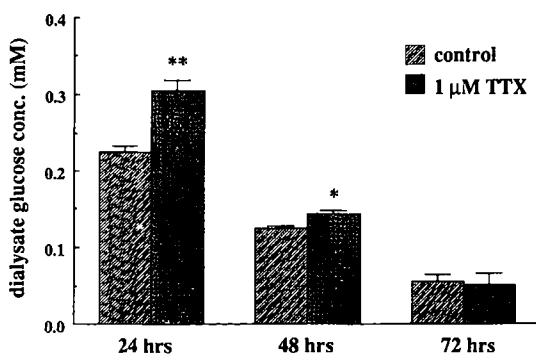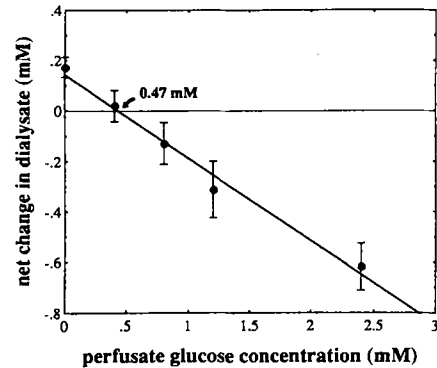

FIG. 3. Determination of the extracellular glucose concentration. The mean net difference between the perfusate glucose concentration introduced into the probe and the recovered concentration in the dialysate is plotted against the perfusate concentration. Data are mean ± SEM (bars) values for five animals.

dialysate glucose efflux was found to be uninfluenced by whether the perfusate concentration had previously been higher or lower than the current level (data not shown).

The concentration of glucose in the ECF was calculated through regression analysis to determine the point where the perfusate and extracellular concentrations were equal and therefore no net change occurred in the dialysate. This gave an estimate of the extracellular glucose concentration as 0.47 ± 0.18 mM (mean ± SEM, n = 5). The slope of the regression line indicates the in vivo recovery of glucose; the mean ± SEM value under these conditions was 33 ± 3.4% ($r^2 = 0.99, p = 0.0007$, n = 5). In all cases, the concentration of glucose in the dialysate when no glucose was added to the perfusate was typical of basal levels measured in the other experiments described in this article.

Glucose levels vary with neuronal activity

To examine the relationship between neuronal activity and local extracellular glucose levels, the sodium channel blocker TTX and the depolarizing agent veratridine were added to the perfusion fluid. The effect of local TTX application is shown in Fig. 4A. The glucose efflux was maximally increased (to 134 ± 7.4% of baseline) at 30 min following drug application and slowly returned toward baseline values despite continuous infusion of the drug. As shown in Fig. 2, the effect of local TTX also changed over time. The increase in dialysate glucose content observed at 24 h diminished over subsequent days. Seventy-two hours following implantation, glucose levels in the presence of TTX were no longer distinguishable from control values.

The local application of veratridine, which opens voltage-gated sodium channels, had the opposite effect on glucose efflux (Fig. 4B). Continuous application of the drug led to a profound (86.3 ± 6.3%) and persistent decrease below basal levels. The animals

FIG. 2. Concentration of glucose in the dialysate under control conditions and in the presence of 1 μM TTX applied through the dialysis probe. Measurements were made in the same animals on the 3 days following implantation. Data are mean ± SEM (bars) values of the last four samples before and the four samples immediately following TTX administration in each of four animals. *p < 0.05, **p < 0.01 compared with controls by paired Student's t test.
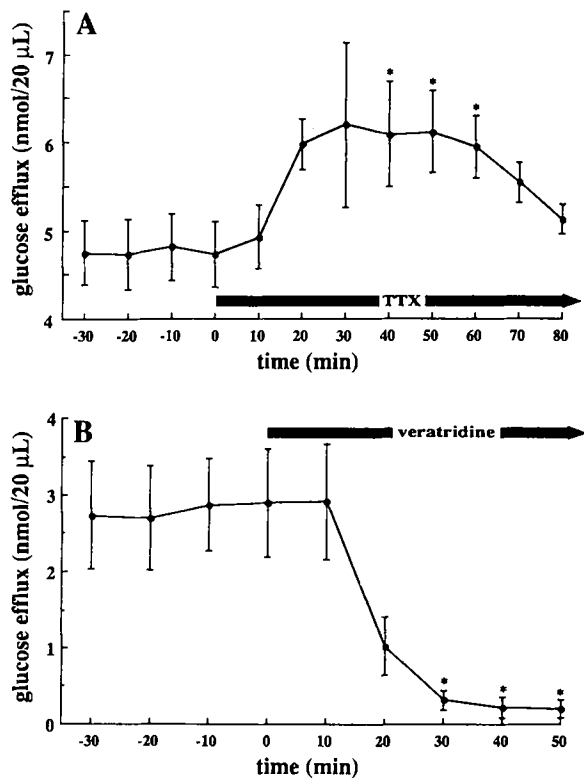
FIG. 4. A: Effect of addition of 1 μM TTX to the perfusion fluid. **B:** Effect of addition of 50 μM veratridine to the perfusion fluid. Data values are mean ± SEM (bars) in four animals in both cases. Both experiments were conducted 24 h following implantation. *p < 0.05 compared with the last predrug point by paired Student's t test.

exhibited no behavioural effects with the application of either drug.

Effects of anaesthesia

In animals implanted 24 h earlier, systemic administration of the general anaesthetic chloral hydrate led to a rapid and sustained increase in the dialysate glucose concentration, reaching levels 250 ± 36% above baseline (Fig. 5). Deep anaesthesia, defined by the absence of the hind limb withdrawal reflex, was established within 10–20 min of the injection. The reflex had not recovered by 100 min postinjection.

DISCUSSION

Previous in vivo studies of extracellular glucose in the brain, using both microdialysis (van der Kuil and Korf, 1991) and voltammetry (Boutelle et al., 1986), found that glucose levels fluctuate under various conditions. This study examined the degree to which extracellular glucose levels are linked to neuronal activity.

Determination of the basal extracellular glucose concentration

We have used microdialysis coupled with the variation-of-concentration method to provide a new tech-

nique for directly determining the extracellular glucose concentration in the awake and unrestrained rat. This gave an estimate of 0.47 mM for the ECF glucose concentration in the striatum.

To compare this value with other estimates, it was important to confirm that the dialysis probe was sampling from normal brain tissue. Disturbances in glucose metabolism have been found immediately following the implantation of microdialysis probes. However, within 2 h the BBB has resealed (Edvinsson et al., 1971; Benveniste and Hüttemeier, 1990), and at 24 h, local cerebral blood flow and glucose utilization in the region around the probe are indistinguishable from control values (Benveniste et al., 1987; Chastain et al., 1990). Neurotransmitter levels measured with microdialysis are most closely linked to neuronal activity at 24 h (Benveniste and Hüttemeier, 1990). Figure 2 shows that dialysate glucose levels were also maximally TTX sensitive at this time. In one other study in which the glucose level was measured with microdialysis, using a different assay, glucose concentrations in the dialysate were found to be similar to those observed in the present experiments (van der Kuil and Korf, 1991).

Chronic microdialysis studies have found that gliosis begins to occur in the vicinity of the implanted probe between 48 and 72 h, with local tissue changes becoming more extensive over time (Benveniste and Hüttemeier, 1990). Such local responses are likely responsible for the decrease in basal dialysate glucose levels and the gradual attenuation of the TTX-stimulated increase seen on subsequent days (Fig. 2).

In the variation-of-concentration method, the net change in the dialysate at a given perfusate glucose concentration reflects an equilibrium between the brain and the dialysate. We have shown that this equilibrium was not influenced by previous levels of glucose in the perfusate, confirming that the point of no net flux reflects the true extracellular concentration and is not an artefact of the experimental protocol.
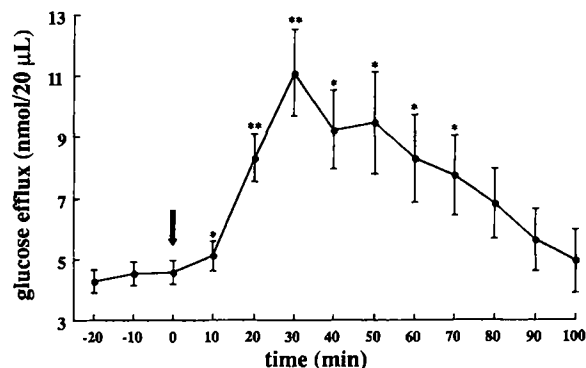

FIG. 5. Effect of the general anaesthetic chloral hydrate (500 mg/kg i.p.) on dialysate glucose levels. The arrow indicates the injection time. Data are mean ± SEM (bars) values in four animals. *p < 0.05, **p < 0.01 compared with the mean baseline by paired Student's t test.

The probe design used in the present experiments gave an in vitro recovery value of 22%. The slightly higher values found for recovery in vivo (mean ± SEM, 33 ± 3.4%) agree with the findings of those studying both neurotransmitter and exogenous drug recovery in vivo (Justice, 1991; Parsons and Justice, 1992). The increase may be due to differences in the concentration gradient in vivo resulting from dynamic transport and uptake processes (Parsons and Justice, 1992).

Comparisons with other estimates

Previously, the extracellular glucose concentration has been calculated from estimations of plasma levels, utilization, and BBB transport kinetics at 2-4 mM (Siesjö, 1978; Lund-Andersen, 1979). These estimates are considerably higher than our measured concentration. It is clear from Fig. 3 that perfusate containing even 0.8 mM glucose loses glucose into the ECF.

The estimated concentration of 2-4 mM was calculated from values for the whole brain determined in anaesthetized rats and neglected nonspecific transport across the BBB. If we substitute more recently obtained values from the striata of awake rats (Cremer et al., 1981; Braun et al., 1985) into the expression used by Lund-Andersen (1979) and correct for nonspecific transport (Braun et al., 1985), a lower estimate of 1.3 mM is obtained. Although this is closer to our measured value, a discrepancy still exists, which suggests that the model of Lund-Andersen (1979) may need to be refined.

Total cerebral glucose content has been measured at 2-4 μmol/g (Siesjö, 1978). Assuming an extracellular volume of 15% and a dry weight of 20% (Lund-Andersen, 1979), substituting an extracellular concentration of 0.47 mM gives an intracellular concentration of 3.4 mM. The cerebral glucose content may be overestimated as a result of plasma contamination. Also, determination of this value frequently involves brief immobilization, which has recently been shown to alter extracellular glucose levels (van der Kuil and Korf, 1991). Nevertheless, these factors are not sufficient to explain the difference between extra- and intracellular concentration.

It has previously been thought that glucose equilibrates rapidly across cell membranes in the brain. This was based on the close agreement between intra- and extracellular concentrations determined when the value of 2 mM for extracellular glucose was substituted as described above (Lund-Andersen, 1979). This agrees with evidence from cultured neurones (Heidenreich et al., 1989). However, there would appear to be a considerable difference between intra- and extracellular concentrations based either on our measured value or on the estimate of extracellular glucose level of 1.3 mM calculated from more recent transport data from awake rats. This suggests that the two pools are not in rapid equilibrium.

Glucose levels vary with drug-induced neuronal activity

The establishment and maintenance of ion gradients constitute a considerable proportion of the brain's energy requirements. The Na^+,K^+-ATPase has been estimated as consuming 35-70% of the brain's metabolic output (Siesjö, 1978; Edwards et al., 1989). TTX and veratridine have opposite effects on the neuronal ion balance. TTX prevents the generation of action potentials by blocking the voltage-gated sodium channel, whereas veratridine causes neuronal depolarization by causing persistent opening of this channel at resting membrane potentials (Catterall, 1984). These two neurotoxins have also been shown to have opposite effects on neuronal energy requirements. In synaptosomal preparations, TTX causes a substantial decrease, whereas veratridine leads to a considerable increase in oxygen consumption (Edwards et al., 1989; Erecińska and Dagani, 1990). Veratridine applied locally in the striatum in concentrations similar to the one used in this study has been shown to elicit substantial release of γ-aminobutyric acid (GABA) (Campbell et al., 1991) and dopamine (Fairbrother et al., 1990) as measured by microdialysis. The depolarization that follows veratridine application may also cause a decrease in extracellular volume as a result of cell swelling (Hansen, 1985). It is not clear whether such changes would lead to an increase in extracellular concentration or to increased tortuosity, leading to decreased recovery by the dialysis probe. The increases in dopamine and GABA levels following veratridine application suggest that any increases in tortuosity do not dramatically alter recovery. Local application of 1 μM TTX leads to a depression of between 55 and 70% in basal dopamine and GABA levels in the striatum (Osborne et al., 1991).

The local application of veratridine resulted in a significant and prolonged decrease in extracellular glucose levels (Fig. 4B). If metabolism were completely limited by transport across the BBB, the extracellular glucose level would tend toward zero. The observation of low glucose concentrations after veratridine application suggests that the increased metabolic rate necessary to fuel the Na^+/K^+ pump succeeds in challenging the rate at which glucose is supplied.

TTX application, which leads to a decrease in local energy requirements, resulted in an increase in extracellular glucose concentration. As the uptake of glucose across the BBB is thought to be closely linked to, if not solely determined by, the rate of glucose phosphorylation (Cremer et al., 1981; Sokoloff, 1981; Hawkins, 1986), this result is somewhat surprising. It would appear that in the awake animal, the link between uptake and utilization is not as close as has been suggested, at least over the short term. Unlike the effect of veratridine, the TTX-induced increase slowly diminished over time, returning to baseline 60 min after TTX was first applied, despite continued infusion of the drug.

As the glucose sampled by the microdialysis probe reflects a balance between supply and utilization, changes in local cerebral blood flow might also play a role in the fluctuations seen with TTX and veratridine. Regional cerebral blood flow is thought to be closely linked to local functional activity, and changes in this parameter have been closely correlated to changes in the local cerebral glucose metabolic rate (Fox et al., 1988). It has been postulated that increases in extracellular K^+ concentration as a result of neuronal activity may regulate regional cerebral blood flow (Paulson and Newman, 1987). These results suggest that local application of TTX would, if anything, decrease local blood flow, whereas veratridine would likely cause an increase. These changes would be expected to have the opposite effects on extracellular glucose concentration to those reported here. This suggests that in the present experiments, extracellular glucose is predominantly being affected by changes in local neuronal metabolic demands, which in turn reflect the intensity of local neuronal activity.

Effects of anaesthesia

Anaesthetics are known to have a profound effect on several aspects of brain metabolism. Under pentobarbital anaesthesia, glucose consumption is approximately halved (Sokoloff et al., 1977), BBB transport is depressed, blood flow diminishes (Pardridge, 1983), and glycogen synthesis is enhanced (Siesjö, 1978). The increase in extracellular glucose level seen in animals under chloral hydrate anaesthesia (Fig. 5) suggests that this anaesthetic also alters the normal glucose balance in the brain. In a separate group of animals, the same dose of chloral hydrate caused a marked increase in plasma glucose levels (authors' unpublished data). The increase in extracellular glucose content may therefore be due in part to changes in the concentration of glucose in the blood.

The parallel decrease in brain glucose transport and utilization observed under pentobarbitol anaesthesia (Sokoloff et al., 1977) has led to the hypothesis that demand and supply are tightly linked under such conditions (Hawkins, 1986). The large increases in extracellular glucose level with animals under chloral hydrate anaesthesia reported here suggest that a general reduction in brain metabolism may not necessarily bring about a well-matched reduction in glucose supply.

The present experiments show that brain glucose transport and utilization are not always closely coupled. Discrepancies between supply and demand occur following both local and global drug-induced changes in neuronal activity. Experiments are now in progress to see whether this also applies under physiological conditions.

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