Extracellular glucose turnover in the striatum of unanaesthetized rats measured by quantitative microdialysis

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1. Steady-state and time-resolved quantitative microdialysis was used to measure dialysate concentration, extracellular concentration and the *in vivo* recovery of glucose in rat striatum.

2. The extracellular concentration of glucose, determined by the zero net flux method of Lönnroth, was 350 ± 20 μM and the *in vivo* recovery was 39 ± 2%.

3. Veratridine caused a steep decrease in dialysate glucose after an initial delay of 7·5 min. When steady-state glucose levels had been reached in the presence of veratridine the extracellular concentration was reduced to zero, but there was no significant change in *in vivo* recovery.

4. Measurement of the dynamic changes during the administration of veratridine showed an immediate decrease in extracellular glucose concentration and a steep rise in *in vivo* recovery, which accounted for the delay in the decrease in dialysate glucose. When extracellular concentration reached zero, *in vivo* recovery returned to control levels.

According to the classical model glucose is delivered to the extracellular compartment of the brain from the blood vascular system; close coupling between neuronal activity and cerebral blood flow suggests that there should be little or no change in extracellular glucose during different levels of glucose utilization (Sokoloff, 1992).

A new model has been proposed, based on recent evidence, in which glucose from the bloodstream is taken up into astrocytes, where it undergoes glycolysis and is then exported as lactate, which serves as a substrate for neuronal energy metabolism (Magistretti & Pellerin, 1996). Since export of glucose has not been demonstrated in astrocytes *in vitro* (Dringen, Gebhardt & Hamprecht, 1993), glucose in the extracellular compartment is assumed to be derived directly from the blood vascular system. However, using microdialysis, we have shown that both drug-induced (Fellows, Boutelle & Fillenz, 1992) and physiologically induced (Fray, Forsyth, Boutelle & Fillenz, 1996) changes in neuronal energy requirements produce changes in dialysate glucose. This casts doubt on the close coupling between supply and utilization (which includes uptake and metabolism) of glucose.

Changes in dialysate concentration are a good index of relative changes in extracellular concentration. However, the relation between the true extracellular concentration and dialysate concentration depends on the *in vivo* recovery. This cannot be derived from the *in vitro* probe recovery because additional factors, dependent on the tissue, play a role in *in vivo* recovery (Bungay, Morrison & Dedrick, 1990; Morrison, Bungay, Hsiao, Ball, Mefford & Dedrick, 1991). These include factors which are constant under physiological conditions, such as the diffusion barriers due to the tortuosity of the extracellular compartment, and others, such as changes in turnover, which fluctuate with neuronal activity (Boutelle & Fillenz, 1996).

A number of techniques have been used to determine the extracellular concentration of analytes. One of these is the zero net flux method of Lönnroth (Lönnroth, Jansson & Smith, 1987). Various concentrations of analyte are added to the perfusion fluid and gain or loss by the brain is plotted against the infused concentration. The point of zero flux across the probe, determined by regression analysis, is the point of equilibrium and is equal to the true extracellular concentration distant from the probe. The slope of the Lönnroth curve measures the *in vivo* recovery of the probe.

Administration of veratridine, which opens the voltage-gated Na^+ channels and so stimulates Na^+–K^+-ATPase, leads to an increase in energy metabolism (Whitesell, Ward, McCall, Granner & May, 1995). In the present study we have used the zero net flux method to monitor both steady-state and dynamic changes in extracellular concentration and *in vivo* recovery of glucose in rat striatum that result from the local infusion of veratridine. The purpose of the study was to determine the normal extracellular concentration of glucose in the striatum of unanaesthetized rats and to investigate changes in turnover rate of extracellular glucose, as measured by *in vivo* recovery, when stimulation of Na^+–K^+-ATPase by veratridine increases metabolic activity.

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METHODS

Surgery

Male Sprague–Dawley rats weighing 200–300 g were anaesthetized, as described previously (Fray *et al.* 1996), with an I.P. injection of 0·25 mg kg^{-1} fentanyl, 0·8 mg kg^{-1} fluanisone and 0·4 mg kg^{-1} midazolam. Surgery typically lasted 35 min and anaesthesia was reversed by an I.P. injection of naloxone (0·1 mg kg^{-1}; Sigma). As animals began to recover from the anaesthetic they were given Vetergesic (Reckitt and Colman Pharmaceuticals, Hull, UK), the long-lasting narcotic partial agonist buprenorphine (0·1 mg kg^{-1}, S.C.), for pain relief.

Fentanyl–fluanisone (Hypnorm) was from Janssen Pharmaceuticals Ltd, Oxford, UK and midazolam (Hypnovel) was from Roche Products Ltd, Welwyn Garden City, UK.

Once surgical anaesthesia was established, animals were placed in a stereotaxic frame and concentric microdialysis probes were implanted in the right striatum, using the following co-ordinates: 1 mm antero-posterior from bregma, 2·5 mm medio-lateral and −8·5 mm below the dura (Paxinos & Watson, 1986). Probes were secured in position using dental screws and acrylic glue (Associated Dental Products Ltd, Swindon, UK).

Animals were allowed 24 h recovery and were assessed for good health, using the guidelines of Morton & Griffiths (1985), after recovery from anaesthesia and at the beginning of the next day. All animals used in this study had a score of 2 or less, as defined by Morton & Griffiths; in cases where the score was 3 or more, the experiment was terminated. This work was carried out under licence in accordance with the Animals (Scientific Procedures) Act, 1986. At the end of the experiment the rats were killed with an overdose of anaesthetic. The position of the probe in the brain was checked post mortem and in all cases the sampling membrane was located entirely within the striatum.

Concentric microdialysis probes were constructed as described previously (Fray *et al.* 1996). The total exposed fibre length was 4 mm and the external diameter of the sampling region of the probe was 300 μm.

Experimental protocol

Following surgery, the animals were placed in large plastic bowls (50 cm × 55 cm) and maintained in a temperature- and light-controlled environment, with free access to food and water. Twenty-four hours after surgery experiments were carried out with the animal in its home bowl, allowing free movement. During experiments the microdialysis probe was perfused with artificial cerebrospinal fluid (ACSF; mM: 147 NaCl, 4 KCl, 1·2 $CaCl_2$ and 1 $MgCl_2$ (de Boer, Damsma, Fibiger, Timmerman, deVries & Westerink, 1990)) at a flow rate of 2 μl min^{-1} using a CMA 100 microinfusion pump (CMA Microdialysis, Stockholm, Sweden). The dialysate was analysed for glucose using a flow injection enzyme-based assay (Boutelle, Fellows & Cook, 1992) together with a pre-oxidation system as described previously (Fray *et al.* 1996). The detection limit of the enzyme bed was 4 pmol, equivalent to 10 μl of 0·4 μM glucose Five microlitre samples were assayed for glucose at 2·5 min intervals.

For the zero net flux method different concentrations of glucose were added to the perfusion medium. The differences between influx and efflux concentrations ([In] − [Out]) were plotted against the infused concentrations; the concentration at which the difference was zero was derived by regression analysis. Results are shown as means ± S.E.M. Statistical significance was calculated by Student's paired *t* test.

RESULTS

We determined the extracellular concentration of glucose and the *in vivo* recovery when three glucose concentrations, 50, 150 and 600 μM, were added to ACSF in random order. Plotting infused concentration against the difference in inflow and outflow ([In] − [Out]) gave a mean extracellular

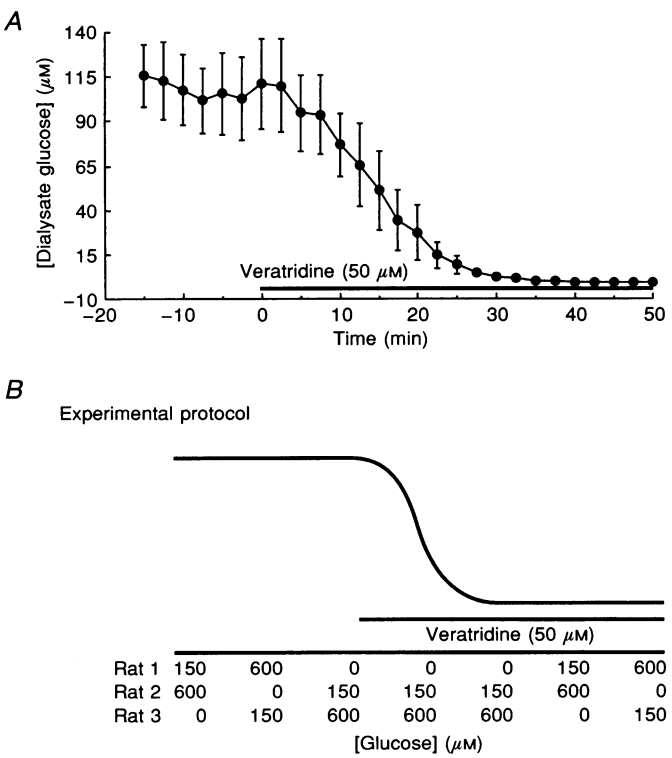

Figure 1. Effect of veratridine on glucose concentration

A, changes in dialysate glucose concentration measured on-line resulting from the addition at 0 time of 50 μM veratridine to the perfusion medium. *B*, experimental protocol for the zero net flux experiment. The upper trace shows a schematic version of the glucose response to veratridine shown in *A*. For each animal there are three phases. (i) The infusion through the probe of 3 different glucose concentrations. (ii) The infusion of 50 μM veratridine in the presence of the last glucose concentration from phase one. (iii) Once a new steady state is achieved the infusion through the probe of 3 different glucose concentrations. In all cases the glucose concentration infused into the probe and the resultant dialysate glucose concentration coming out of the probe are measured with the on-line assay. The differences between infused and dialysate glucose concentrations are then plotted against the infused glucose concentration to give a series of zero net flux plots. For phases (i) and (iii) each plot has data from the same animal; for phase (ii) plots are created at each time point using data from all three animals.

glucose concentration of 350 ± 20 μM and a mean *in vivo* recovery for glucose of 39 ± 2% ($n = 15$) under control conditions.

We next investigated the effect of the infusion of veratridine. Figure 1*A* shows the change in dialysate glucose concentration resulting from the local infusion through the dialysis probe of 50 μM veratridine. A steep decrease occurred after a delay of about 7·5 min, in spite of the instant and potent effect of veratridine on energy consumption in the brain. This was not due to delays in either the drug reaching the brain or the dialysate reaching the detector.

In order to determine the effects of veratridine on extracellular glucose concentration and *in vivo* recovery, a zero net flux experiment was carried out with normal perfusion medium followed by perfusion medium containing 50 μM veratridine. The total duration of such an experiment was 6 h. In order to exclude time-dependent changes, two successive zero net flux experiments were carried out in the absence of drug. There was no difference in either extracellular concentration or recovery between the two sequential experiments (not shown).

When a zero net flux experiment under control conditions was followed by one in which the perfusion fluid contained 50 μM veratridine, the dialysate glucose concentration was reduced from a control value of 114 ± 6 to 0·4 ± 7 μM ($P = 0·001$), the extracellular concentration from 309 ± 32 to −16 ± 12 μM ($P = 0·002$) and *in vivo* recovery increased from 38 ± 3 to 52 ± 6% ($P = 0·133$, $n = 5$) (Fig. 2).

These data confirm that there is a fall in the steady-state extracellular concentration of glucose after a 6 h infusion of veratridine, but they fail to explain the delay in the decrease of the dialysate concentration. In order to follow the *dynamic* changes during the administration of veratridine, three rats were perfused with different concentrations of glucose when veratridine was first added to the perfusion medium. The protocol of the experiment is shown in Fig. 1*B*. Regression curves were constructed for the measurements carried out at 2·5 min intervals. To validate this procedure a comparison of the means of within-animal zero net flux experiments with between-animal zero net flux experiments is shown in Fig. 3*A*.

Figure 3*B* shows the last regression curve with drug-free perfusion medium and the first five regression curves immediately following the addition of veratridine to the perfusion medium. The first three regression curves after addition of veratridine show a progressive increase in slope, indicating an increase in *in vivo* recovery. This results in a change in extracellular concentration, but no change in dialysate concentration. The next regression curve shows little further change in slope but a move to the left which produces a change in both extracellular and dialysate concentration. After this there is a decrease in recovery as well as a move to the left which leads to a parallel reduction in extracellular concentration and dialysate concentration.

The full time course of the changes in calculated extracellular concentration and measured dialysate concentration are shown in Fig. 4. Both extracellular concentration and

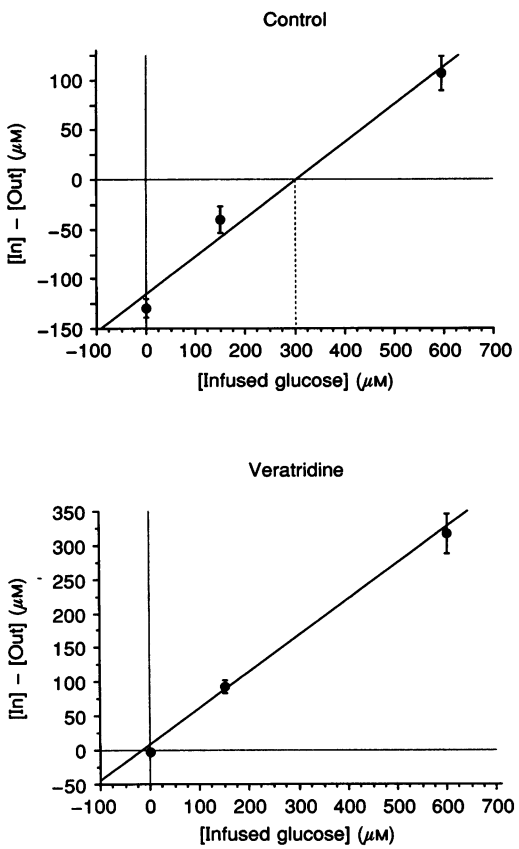

Figure 2. Regression curves constructed from zero net flux experiments during infusion of normal perfusion medium (control) and during perfusion with medium containing 50 μM veratridine

Zero net flux across the probe membrane is when [In] − [Out] = 0; this represents the extracellular concentration of glucose. The slope of the curve is the *in vivo* recovery, which is a property of the probe and the tissue surrounding the probe. Veratridine affects both the extracellular fluid concentration and the *in vivo* recovery.

dialysate concentration fall to a very low level, but the decrease in extracellular concentration is immediate whereas the decrease in dialysate is delayed. This is shown more clearly if both extracellular and dialysate changes are shown as percentage changes of glucose concentration. The disparity in time course between extracellular and dialysate concentrations is explained by the change in *in vivo* recovery. The latter shows an immediate increase from a control value of 28% to a maximum of 59% within the first 7·5 min. The effect on dialysate concentration of the increase in *in vivo* recovery is to counteract the decrease in extracellular concentration. Having reached a peak at 10 min the *in vivo* recovery begins to decrease and reaches a steady level of 37%.

DISCUSSION

Most previous estimates of the extracellular concentration of glucose are based on calculations from total brain content, which neglect the presence of astrocytes with their glycogen content (Pardridge, 1983). In the present study we have used the zero net flux method of Lönnroth; the value of 350 μM, based on fifteen separate experiments, is very similar to our previous figure of 470 μM derived from five determinations (Fellows *et al.* 1992) and the value reported by Langemann and her colleagues (Langemann, Alessandri, Landolt, Bächli & Grazl, 1996). In a separate study using an implanted biosensor and a modified Lönnroth procedure, we obtained a value of 335 μM (Lowry & Fillenz, 1996). In another study, which used anaesthetized rats and in which two concentrations of glucose were infused into the two striata of the same rat, the value obtained for the extracellular glucose concentration was 3300 μM (Ronne-Engström, Carlson, Yansheng, Ungerstedt & Hillered, 1995). The use of an implanted glucose oxidase-based sensor in the hypothalamus of anaesthetized rats gave a value of 2·4 mM (Silver & Erecinska, 1994). The reason for these differences is not clear at present.

With the zero net flux method the true extracellular concentration of glucose at a distance from the probe can be determined (Boutelle & Fillenz, 1996). This concentration reflects the balance between supply to the extracellular fluid and utilization. The method also measures the *in vivo* recovery of the probe, which relates the dialysate concentration to the extracellular concentration. The *in vivo* recovery varies with properties of the probe and tissue factors. So long as the physical state of the tissue is constant the only factors that can affect recovery are changes in release and uptake of the compound being measured. Thus an increase in utilization will increase the *in vivo* recovery and hence the measured slope. In this way the *in vivo* recovery can be used as an index of turnover (Morrison *et al.* 1991). Changes in *in vivo* recovery as an index of changes in turnover rate of dopamine resulting from 6-OH dopamine lesions or inhibition of dopamine uptake and metabolism have been demonstrated by Justice (1993).

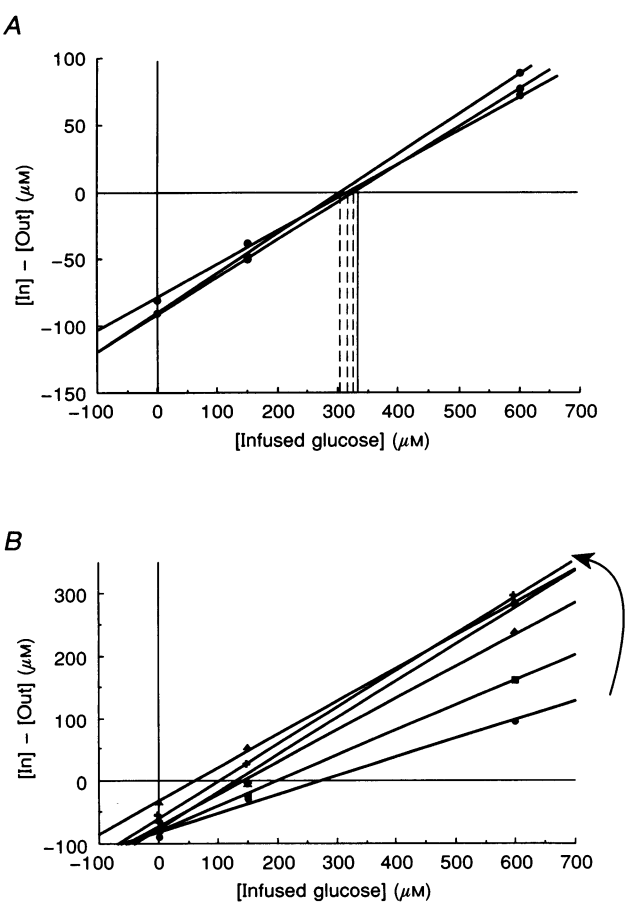

Figure 3. Dynamic changes in extracellular glucose concentration after administration of 50 μM veratridine

A, method validation. A comparison of within- and between-animal regression curves for the control period of zero net flux experiment using three rats (data from Fig. 1*B*, phase (i)). The dashed lines are from the three individual rats and the continuous line is the mean from the three rats. *B*, six sequential regression curves from Fig. 1*B*, phase (ii). Measurements are at 2·5 min intervals, starting with the last regression curve during the control period and followed by the first five regression curves after the addition of veratridine to the perfusion medium with increasing time indicated by arrow. Addition of veratridine leads to an initial increase followed by a decrease in slope and thus *in vivo* recovery.

Veratridine, which opens voltage-gated Na^+ channels, is a powerful stimulant to Na^+–K^+-ATPase, and therefore energy consumption. In spite of this there is a delay in the fall of glucose using simple microdialysis. In order to follow the effects of increased glucose utilization on both the extracellular concentration and *in vivo* recovery we have used the zero net flux technique.

With the steady-state technique there is a profound decrease in extracellular glucose concentration, which is also reflected in the reduction in the dialysate concentration; the increase in *in vivo* recovery, however, is not statistically significant. This shows, perhaps surprisingly, that at this stage there is no significant difference in the rate of glucose utilization.

The time-resolved technique allows us to follow the dynamic effects of veratridine. The addition of veratridine leads to both doubling of *in vivo* recovery, and a parallel steep decline in extracellular glucose concentration. This signals a large increase in glucose utilization, triggered by veratridine, and the resulting depletion of extracellular glucose. The increase in *in vivo* recovery compensates for the decrease in extracellular glucose concentration and explains the delayed fall in dialysate concentration. Having reached a peak, the *in vivo* recovery begins to return close to control values. This occurs when extracellular glucose concentrations have fallen to a very low level. We interperet this as a failure in the rate of glucose utilization due to the very low extracellular glucose level.

The present experiments have a number of implications. The normal low extracellular concentration implies that extracellular glucose is not derived directly from the blood vascular system and supports the hypothesis of a compartmentation of brain metabolism (Forsyth, Fray, Boutelle, Fillenz, Middleditch & Burchell, 1996; Magistretti & Pellerin, 1996). This is further supported by the finding that, in spite of the steep concentration gradient between plasma and the rapidly decreasing extracellular concentration resulting from the infusion of veratridine, supply is unable to compensate for the rapid depletion. Because of the opposite effect of glucose utilization on *in vivo* recovery and extracellular concentration, dialysate concentrations are no longer a reliable index of changes in extracellular glucose concentration during rapid changes in utilization.

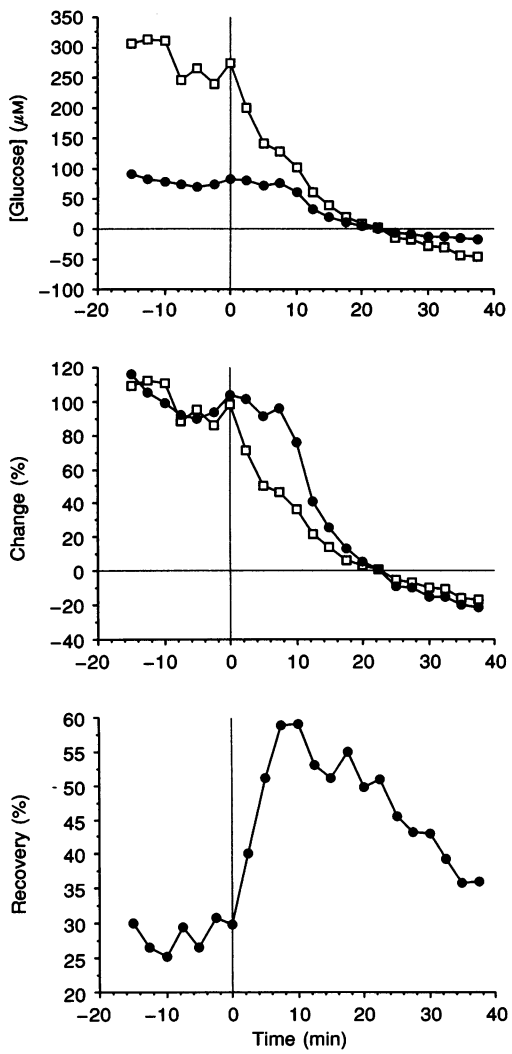

Figure 4. Comparison of the time courses of changes in calculated extracellular and measured dialysate glucose concentrations

Veratridine (50 μM) was added to the perfusion medium at 0 time. Concentrations of glucose are shown in absolute units and as percentage change with respect to concentration at 0 time in the upper and middle panels, respectively (□, extracellular and ●, dialysate glucose concentrations); the bottom panel shows the changes in *in vivo* recovery.

In conclusion, the effects of veratridine on extracellular glucose turnover can be interpreted as follows: stimulation of Na^+–K^+-ATPase leads to an immediate and high rate of utilization, which is dependent on the availability of glucose; when that is exhausted, utilization also declines.

Boutelle, M. G., Fellows, L. K. & Cook, C. (1992). Enzyme packed bed system for the on-line measurement of glucose, glutamate and lactate in brain microdialysate. *Analytical Chemistry* **64**, 1790–1794.

Boutelle, M. G. & Fillenz, M. (1996). Clinical microdialysis: The role of on-line measurement and quantitative microdialysis. *Acta Neurochirurgica*, suppl. 67, 13–20.

Bungay, P. M., Morrison, P. F. & Dedrick, R. L. (1990). Steady-state theory for quantitative microdialysis of solutes and water *in vivo* and *in vitro*. *Life Sciences* **46**, 105–119.

de Boer, J., Damsma, G., Fibiger, H. C., Timmerman, N., deVries, J. B. & Westerink, B. H. C. (1990). Dopaminergic–cholinergic interactions in the striatum: the critical significance of Ca^{2+} concentration in brain microdialysis. *Naunyn–Schmiedeberg's Archives of Pharmacology* **342**, 528–534.

Dringen, R., Gebhardt, R. & Hamprecht, B. (1993). Glycogen in astrocytes: Possible function as lactate supply for neighboring cells. *Brain Research* **623**, 208–214.

Fellows, L. K., Boutelle, M. G. & Fillenz, M. (1992). Extracellular brain glucose levels reflect local neuronal activity: a microdialysis study in awake, freely moving rats. *Journal of Neurochemistry* **59**, 2141–2147.

Forsyth, R., Fray, A., Boutelle, M., Fillenz, M., Middleditch, C. & Burchell, A. (1996). A role for astrocytes in glucose delivery to neurons? *Developmental Neuroscience* **18**, 360–370.

Fray, A. E., Forsyth, R. J., Boutelle, M. G. & Fillenz, M. (1996). The mechanisms controlling physiologically stimulated changes in rat brain glucose and lactate: a microdialysis study. *Journal of Physiology* **496**, 49–57.

Justice, J. B. J. (1993). Quantitative microdialysis of neurotransmitters. *Journal of Neuroscience Methods* **48**, 263–276.

Langemann, H., Alessandri, B., Landolt, H., Bächli, H. & Grazl, O. (1996). True levels of glucose and lactate measured by microdialysis in the cerebral extracellular fluid of patients. In *Monitoring Molecules in Neuroscience*, ed. Gonzales-Mora, J. L., Borges Rand, R. & Mas, M., pp. 397–399. University of La Laguna, Santa Cruz de Tenerife, Spain.

Lönnroth, P., Jansson, P.-A. & Smith, U. (1987). A microdialysis method allowing characterization of intercellular water space in humans. *American Journal of Physiology* **253**, E228–231.

Lowry, J. P. & Fillenz, M. (1996). *In vivo* glucose measurements in rat striatum with an implanted sensor. *Journal of Neurochemistry* **66**, S92A.

Magistretti, P. J. & Pellerin, L. (1996). Cellular bases of brain energy metabolism and their relevance to functional brain imaging: Evidence for a prominent role of astrocytes. *Cerebral Cortex* **6**, 50–61.

Morrison, P., Bungay, P., Hsiao, J., Ball, B., Mefford, I. & Dedrick, R. (1991). Quantitative microdialysis: analysis of transients and application to pharmacokinetics in brain. *Journal of Neurochemistry* **57**, 103–119.

Morton, D. B. & Griffiths, P. H. M. (1985). Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. *Veterinary Record* **116**, 431–436.

Pardridge, W. M. (1983). Brain metabolism: a perspective from the blood brain barrier. *Physiological Reviews* **63**, 1481–1535.

Paxinos, G. & Watson, C. (1986). *The Rat Brain in Stereotaxic Coordinates*. Academic Press, Sydney.

Ronne-Engström, E., Carlson, H., Yansheng, L., Ungerstedt, U. & Hillered, L. (1995). Influence of perfusate glucose concentration on dialysate lactate, pyruvate, aspartate, and glutamate levels under basal and hypoxic conditions: A microdialysis study in rat brain. *Journal of Neurochemistry* **65**, 257–262.

Silver, A. & Erecinska, M. (1994). Extracellular glucose concentration in mammalian brain: continuous monitoring of changes during increased neuronal activity and upon limitation in oxygen supply in normo-, hypo-, and hyperglycaemic animals. *Journal of Neuroscience* **14**, 5068–5076.

Sokoloff, L. (1992). The brain as a chemical machine. *Progress in Brain Research* **94**, 19–33.

Whitesell, R. R., Ward, M., McCall, A. L., Granner, D. K. & May, J. M. (1995). Coupled glucose transport and metabolism in cultured neuronal cells: Determination of the rate-limiting step. *Journal of Cerebral Blood Flow and Metabolism* **15**, 814–826.

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