

PHYSIOLOGICAL SOCIETY SYMPOSIUM: GLUCOSE TRANSPORT: A FUNCTIONAL APPROACH

THE RELATION BETWEEN LOCAL CEREBRAL BLOOD FLOW AND EXTRACELLULAR GLUCOSE CONCENTRATION IN RAT STRIATUM

MARIANNE FILLENZ* AND JOHN P. LOWRY†

University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, UK

SUMMARY

The effect of anaesthetics on the relation between local cerebral blood flow (rCBF) and extracellular glucose was studied in rat striatum. Cerebral blood flow was measured using the hydrogen clearance method and extracellular glucose using an implanted glucose oxidase-based biosensor. Rats were given an intraperitoneal (i.P.) injection of either sodium pentobarbitone (60 mg kg^{-1}) or chloral hydrate (350 mg kg^{-1}). The effect of the i.P. injection, as demonstrated by an i.P. saline injection, was a brief increase in rCBF accompanied by a decrease in glucose. Sodium pentobarbitone produced a decrease in both rCBF and glucose, whereas chloral hydrate caused a decrease in glucose but an increase in rCBF. These findings show a dissociation between rCBF and extracellular glucose and suggest that glucose in the extracellular compartment is not derived directly from the blood vascular compartment.

INTRODUCTION

Glucose is the sole metabolic substrate for the brain in normal adult animals. According to the classical model glucose is supplied to the extracellular compartment directly from the cerebro-vascular compartment; changing energy requirements in this model are met by changes in local cerebral blood flow and there is close coupling between glucose delivery and local cerebral blood flow (Sokoloff, 1992; Jueptner & Weiller, 1995).

Recent evidence suggests that astrocytes have a central role in the delivery of metabolic substrates. Anatomical studies have shown that astrocytes have processes which envelop the brain capillaries and the synaptic complexes and so intervene between the vascular compartment and neurones (Wolff, 1970). In a preparation of mammalian retina consisting of glial Müller cells and photoreceptors, labelled glucose is taken up exclusively into the glial cells, which then export lactate; this is taken up by the photoreceptors (Poiry-Yamate, Poiry & Tsacopoulos, 1995). These findings have led to the hypothesis that brain metabolism is compartmentalized, that glucose taken up by astrocytes undergoes aerobic glycolysis and the resulting lactate is exported and taken up by neurones as their source of energy (Magistretti & Pellerin, 1996).

* Corresponding author: fillenz@physiol.ox.ac.uk

† Present address: Department of Chemistry, University College Dublin, Belfield, Dublin 4, Ireland.

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Support for this model has come from *in vivo* experiments. The use of quantitative microdialysis has shown that in unanaesthetized rats there is a basal lactate concentration of 350 µM (Demestre, Boutelle & Fillenz, 1997); stimuli which lead to neuronal activation, such as tail pinch or restraint, lead to an increase in dialysate lactate (Fellows, Boutelle & Fillenz, 1993; Fray, Forsyth, Boutelle & Fillenz, 1996); the demonstration of lactate export by astrocytes *in vitro* (Dringen, Wiesinger & Hamprecht, 1993; Pellerin & Magistretti, 1994) suggests that astrocytes may be the source of this lactate increase. Since export of glucose from astrocytes has not been demonstrated *in vitro*, it has been assumed that glucose enters the extracellular compartment directly from the bloodstream (Magistretti & Pellerin, 1996). A number of findings from *in vivo* experiments are in conflict with such a view.

Calculations of extracellular concentration of glucose in the brain derived from the glucose content and the size of the extracellular compartment have produced figures for the extracellular concentration of 2.0 mM (Lund-Anderson, 1979). These calculations have ignored the astrocytic compartment, which contains glycogen as a possible source of glucose. Determination of extracellular glucose concentration using quantitative microdialysis has shown that the extracellular concentration is 0.35 µM (Fray, Boutelle & Fillenz, 1997). A similar figure has been obtained using an implanted glucose sensor (Lowry & Fillenz, 1996; Lowry, O'Neill, Boutelle & Fillenz, 1998). Such a low concentration argues against the view that glucose is derived directly from the bloodstream.

Even stronger evidence comes from the measurement of the effect of neuronal activation on changes in dialysate glucose. Stimuli which lead to activation of rat striatum, such as tail pinch, lead to changes in both local cerebral blood flow and extracellular glucose. But whereas the increase in rCBF is immediate and co-extensive with the neuronal activation, the increase in dialysate glucose is delayed and very much more prolonged (Fellows, Boutelle & Fillenz, 1993). More recently we have shown that the rise in glucose is preceded by a decrease in dialysate glucose which is co-extensive with neuronal activation (Fray *et al.* 1996). The development of implanted sensors for glucose and for oxygen enables continuous recording of changes in these two parameters. In rats implanted with a platinum electrode in one striatum, for measurement of rCBF by the hydrogen clearance method, and a carbon paste electrode in the other striatum, for measurement of changes in tissue oxygen, studies have shown an increase in rCBF and tissue oxygen during neuronal activation. The magnitude of the increase in tissue oxygen is similar to the increase in rCBF and is a reflection of the latter; there is little or no evidence for an increase in oxygen utilization. Glucose, on the other hand, decreases during the period of stimulation, which suggests an increase in utilization, since rCBF is increased. At the end of stimulation there is a slow and prolonged increase in glucose. Since by this time rCBF has returned to control level the rise in glucose cannot be derived directly from the bloodstream (Lowry & Fillenz, 1997).

In the present study we have examined the relation between changes in rCBF and extracellular glucose when neuronal activity is altered by the administration of anaesthetics.

METHODS

Male Sprague-Dawley rats weighing 200–300 g were anaesthetized as described previously (Fray *et al.* 1996; Lowry & Fillenz, 1997).

Once surgical anaesthesia was established animals were placed in a stereotaxic frame. In one group of rats a platinum/poly(*o*-phenylenediamine)polymer/glucose oxidase (Pt/PPD/GOx) electrode was implanted in the right striatum and in another group a 2T platinum/iridium electrode was implanted in the right striatum as described previously (Lowry & Fillenz, 1997).

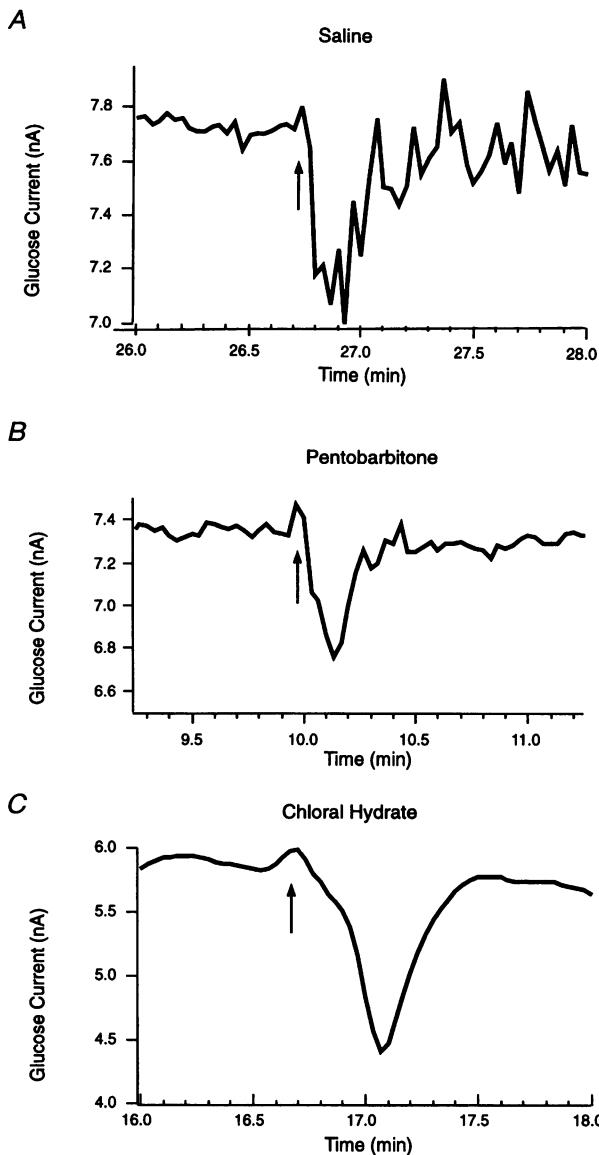


Fig. 1. Changes in glucose current in response to the intraperitoneal injection of saline (A), sodium pentobarbitone (B) or chloral hydrate (C). Arrows indicate the points of injection.

Animals were assessed for good health using the guidelines of Morton & Griffiths (1985) after recovery from anaesthesia and at the beginning of each 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.

Regional cerebral blood flow measurements were carried out at 5 min intervals as described in previous publications (Fellows & Boutelle, 1993; Lowry & Fillenz, 1997).

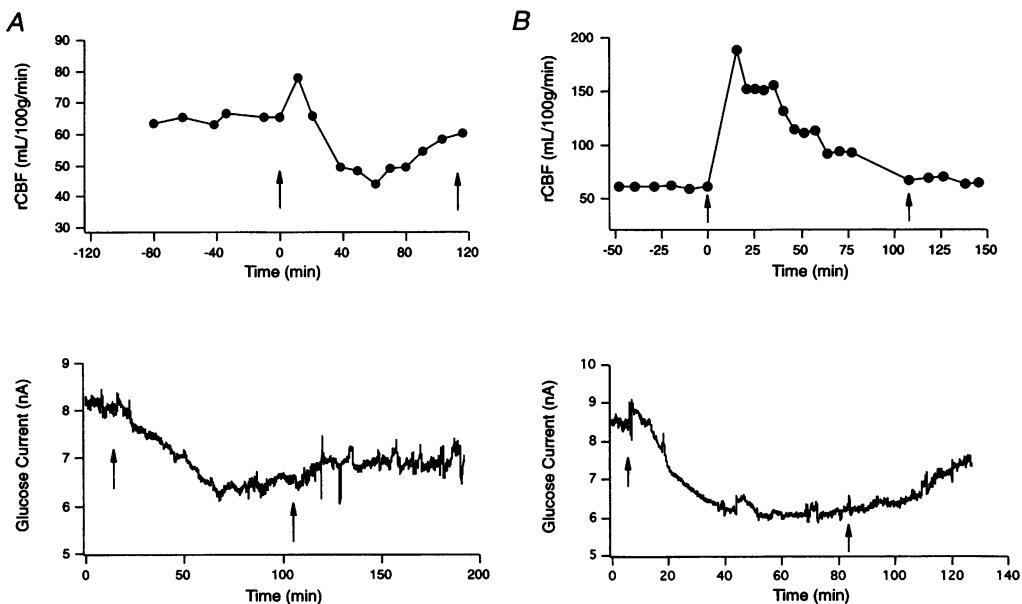


Fig. 2. Changes in local cerebral blood flow (rCBF; in $\text{ml} (\text{100 g})^{-1} \text{ min}^{-1}$) and glucose current (nA) in response to the intraperitoneal injection of 60 mg kg^{-1} sodium pentobarbitone (A) or 400 mg kg^{-1} chloral hydrate (B). Arrows indicate the point of injection and the end of anaesthesia.

Rats were given an intraperitoneal (I.P.) injection of either sodium pentobarbitone (60 mg kg^{-1}) or chloral hydrate (400 mg kg^{-1}). The level of anaesthesia was monitored by the use of the palpebral reflex and the hindlimb withdrawal reflex.

RESULTS

Effect of saline injection

Since the anaesthetics were administered by intraperitoneal injection we first examined the effect of an I.P. injection of saline. There was an increase in only the first of the 5 min rCBF measurements after the injection and a return to baseline by the next one. The effect of the injection on glucose on the other hand was a very brief decrease (Fig. 1A), with a mean duration of $0.74 \pm 0.31 \text{ min}$ ($n = 3$).

Effect of sodium pentobarbitone

The I.P. injection of 60 mg kg^{-1} sodium pentobarbitone produced the same initial effect on rCBF and glucose (Fig. 1B) as the saline injection. This was followed by a gradually deepening anaesthesia with a mean duration of $92.2 \pm 10.8 \text{ min}$ ($n = 5$). The initial increase in rCBF due to injection was quickly followed by a decrease to $71 \pm 5\%$ of basal which was reached $42.05 \pm 3.85 \text{ min}$ after the injection. There was a similar but slower decrease in glucose to $74 \pm 6\%$ of control by $60.22 \pm 12.08 \text{ min}$ after the injection ($n = 3$). As the rats showed signs of recovery from the anaesthetic there was a slow recovery of both the rCBF and the level of glucose, which, in spite of behavioural recovery from the anaesthetic, had not

reached control levels 2 h after the administration of the anaesthetic. A typical example is shown in Fig. 2A.

Effect of chloral hydrate

The I.P. injection of 400 mg kg^{-1} chloral hydrate produced anaesthesia with a mean duration of $69 \pm 7 \text{ min}$ ($n = 10$). After the initial effect of the injection (Fig. 1C) glucose showed a slow decrease which reached $74 \pm 5\%$ of control $85.27 \pm 9.55 \text{ min}$ after the injection ($n = 5$). In contrast to sodium pentobarbitone, after chloral hydrate rCBF increased to $267 \pm 59\%$ of control at $17.97 \pm 2.38 \text{ min}$ followed by a slow decrease in spite of continued anaesthesia ($n = 5$). A characteristic example is shown in Fig. 2B.

DISCUSSION

The purpose of the present study was to examine the relation between rCBF and glucose in the extracellular compartment of the brain. The concentration of extracellular glucose represents the balance between supply and utilization. The implanted glucose biosensor, in contrast to microdialysis, provides a technique for continuous monitoring of extracellular glucose concentration. The results show a dissociation between rCBF and extracellular glucose.

The stress of an I.P. injection stimulates neuronal activity as shown by the activation of the serotonergic and noradrenergic projection to the hippocampus (Vahabzadeh & Fillenz, 1994). The brief decrease in glucose together with the increase in rCBF which accompanies the I.P. injection therefore suggests an increase in neuronal glucose utilization due to neuronal activation.

A comparison of the effects of chloral hydrate and pentobarbitone anaesthesia in rats found a lowering of blood pressure with both anaesthetics (Field, White & Lang, 1993). An earlier study showed that barbiturates produce a parallel reduction in cerebral blood flow and oxygen consumption (Siesjö, 1978). Since under basal conditions there is a close correlation between cerebral consumption of oxygen and glucose, the reduced oxygen consumption suggests that barbiturates will also reduce glucose consumption. Glucose consumption in turn has been shown to vary with neuronal activity (Jueptner & Weiller, 1995). The reduction in extracellular glucose after pentobarbitone in the present study must reflect decreased delivery rather than increased utilization.

The changes following chloral hydrate are more difficult to interpret. It is an anaesthetic used largely in animal experiments and there is information on the effects on EEG. With some anaesthetics there is a transitional phase of excitation; although there is no behavioural evidence for such an effect with chloral hydrate, the transient increase in rCBF suggests that this may be the case. Furthermore microdialysis experiments have shown that chloral hydrate causes an increase in dopamine release in rat striatum (Petrinec, Guadalupe, Fumero, Virjo, Gonzales-Mora & Mas, 1996). The decrease in extracellular glucose has a very different time course from the rCBF changes and could reflect both increased utilization and decreased delivery.

Whatever the detailed mechanisms of the changes in glucose, the dissociation between these and rCBF suggest that glucose must come from a compartment other than the blood vascular system.

REFERENCES

- DEMESTRE, M., BOUTELLE, M. G. & FILLENZ, M. (1997). Stimulated release of lactate in freely moving rats is dependent on the uptake of glutamate. *Journal of Physiology* **499**, 825–832.
- DRINGEN, R., WIESINGER, H. & HAMPRECHT, B. (1993). Uptake of L-lactate by cultured rat brain neurons. *Neuroscience Letters* **163**, 5–7.
- FELLOWS, L. K. & BOUTELLE, M. G. (1993). Rapid changes in extracellular glucose levels and blood flow in the striatum of the freely moving rat. *Brain Research* **604**, 225–231.
- FELLOWS, L. K., BOUTELLE, M. G. & FILLENZ, M. (1993). Physiological stimulation increases non-oxidative glucose metabolism in the brain of the freely moving rat. *Journal of Neurochemistry* **60**, 1258–1263.
- FIELD, K. J., WHITE, W. J. & LANG, C. M. (1993). Anaesthetic effects of chloral hydrate, pentobarbitone and urethane in adult male rats. *Laboratory Animals* **27**, 258–269.
- FRAY, A. E., BOUTELLE, M. G. & FILLENZ, M. (1997). Extracellular glucose turnover in the striatum of unanaesthetized rats measured by quantitative microdialysis. *Journal of Physiology* **504**, 721–726.
- 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.
- JUEPTNER, M. & WEILLER, C. (1995). Does measurement of regional cerebral blood flow reflect synaptic activity? Implications for PET and fMRI. *Neuroimage* **2**, 148–156.
- LOWRY, J. P. & FILLENZ, M. (1996). In vivo glucose measurements in rat striatum with an implanted sensor. *Journal of Neurochemistry* **66**, S92A.
- LOWRY, J. P. & FILLENZ, M. (1997). Evidence for uncoupling of oxygen and glucose utilization during neuronal activation in rat striatum. *Journal of Physiology* **498**, 497–501.
- LOWRY, J. P., O'NEILL, R. D., BOUTELLE, M. G. & FILLENZ, M. (1998). Continuous monitoring of extracellular glucose concentrations in the striatum of freely moving rats with an implanted glucose biosensor. *Journal of Neurochemistry* (in the Press).
- LUND-ANDERSON, H. (1979). Transport of glucose from blood to brain. *Physiological Reviews* **59**, 305–352.
- 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.
- MORTON, D. B. & GRIFFITHS, P. H. M. (1985). Guidelines on the recognition of pain, distress and discomfort in experimental animals and a hypothesis for assessment. *Veterinary Record* **116**, 431–436.
- PELLERIN, L. & MAGISTRETTI, P. J. (1994). Glutamate uptake into astrocytes stimulates aerobic glycolysis: A mechanism coupling neuronal activity to glucose utilisation. *Proceedings of the National Academy of Sciences of the USA* **91**, 10625–10629.
- PETRINEC, J., GUADALUPE, P. J., FUMERO, B., VIRJO, E., GONZALES-MORA, J. L. & MAS, M. (1996). Effects of different anaesthetics on striatal dopaminergic activity as assessed by in vivo voltammetry. In *Monitoring Molecules in Neuroscience*, ed. GONZALES-MORA, J. L., BORGES, R. & MAS, M., pp. 293–294. University of La Laguna, Santa Cruz de Tenerife, Spain.
- POITRY-YAMATE, C. L., POITRY, S. & TSACOPOULOS, M. (1995). Lactate released by Müller glial cells is metabolized by photoreceptors from mammalian retina. *Journal of Neuroscience* **15**, 5179–5191.
- SIESJÖ, B. K. (1978). *Brain Energy Metabolism*. John Wiley & Sons, Chichester, UK.
- SOKOLOFF, L. (1992). The brain as a chemical machine. *Progress in Brain Research* **94**, 19–33.
- VAHABZADEH, A. & FILLENZ, M. (1994). Comparison of stress-induced changes in noradrenergic and serotonergic neurons in the rat hippocampus using microdialysis. *European Journal of Neuroscience* **6**, 1205–1212.
- WOLFF, J. R. (1970). The astrocyte as link between capillary and nerve cell. *Triangle* **9**, 153–164.