Evidence for uncoupling of oxygen and glucose utilization during neuronal activation in rat striatum

John P. Lowry and Marianne Fillenz*

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

1. Changes in regional cerebral blood flow (rCBF), tissue oxygen and extracellular glucose were measured during neuronal activation, using implanted electrodes in the striatum of freely moving rats.

2. There was a parallel increase in rCBF and oxygen in response to neuronal activation.

3. During the neuronal activation there was a decrease in extracellular glucose; following neuronal activation there was a slow rise in extracellular glucose which took 30 min to return to basal levels.

4. The implications of the different time courses of these changes are discussed.

Neuronal activity depends on ion gradients which are maintained by energy-requiring active transport. For this the brain requires a constant supply of glucose and oxygen, which are delivered by the cerebral circulation. Using positron emission tomography a close correlation between global oxygen and glucose consumption and cerebral blood flow was found under normal conditions in human subjects (Baron, Lebrun-Grandie, Collard, Crouzel, Mestelan & Bousser, 1982). Complete oxidation of glucose to CO_2 and H_2O would give a ratio of oxygen to glucose consumption of 6 : 1; the measured ratio is 5·5, which may be partly due to the fact that glucose has functions other than energy metabolism. The use of double tracer techniques for the measurement of regional cerebral blood flow (rCBF) and regional cerebral glucose metabolism (rCM_{glc}) has shown that they are closely coupled and vary in parallel with neuronal activity (Sokoloff, 1992; Jueptner & Weiller, 1995). Studies using inhalation of radio-labelled oxygen have shown an uncoupling of rCM_{glc} and regional cerebral oxygen metabolism (rCM_{O_2}) during brief increases in neuronal activity (Fox & Raichle, 1986; Fox, Raichle, Mintun & Dence, 1988).

With the application of the techniques of microdialysis and hydrogen clearance in animal experiments it has been possible to monitor the time course of these changes. Such experiments have shown that there is a very rapid increase in rCBF, a slightly slower increase in lactate and a delayed and more prolonged rise of glucose in the extracellular fluid (Fellows & Boutelle, 1993; Fellows, Boutelle & Fillenz, 1993). Furthermore, the increases in rCBF, lactate and glucose are controlled by different mechanisms (Fray, Forsyth, Boutelle & Fillenz, 1996). This implies that lactate and glucose in the extracellular fluid are not directly derived from the blood vascular system and supports the hypothesis of metabolic compartmentation with astrocytes playing an important role in the supply of metabolic substrates for neuronal metabolism (Tsacopoulos & Magistretti, 1996).

In the present study we have measured rCBF, tissue oxygen and extracellular glucose in the striatum of freely moving rats during neuronal activation. Glucose and oxygen were measured with implanted electrodes which give a continuous record and so provide the true time course of these changes. This has enabled us to examine the relation between glucose and oxygen utilization.

METHODS

Surgical procedures

Male Sprague–Dawley rats weighing 200–300 g were anaesthetized (following published guidelines, Wolfensohn & Lloyd, 1994) with the following mixture: Hypnorm (Janssen Pharmaceuticals Ltd, Oxford, UK), a combination of the neuroleptic analgesic fentanyl (0·318 mg ml^{-1}) and fluanisone (10 mg ml^{-1}); Hypnovel (Roche), the benzodiazepine midazolam (5 mg ml^{-1}); and sterile water; mixed in the ratio 1 : 1 : 2. This mixture was injected at a volume of 3·3 ml kg^{-1} I.P., giving a dose of 0·25 mg kg^{-1} fentanyl, 0·8 mg kg^{-1} fluanisone and 0·4 mg kg^{-1} midazolam. Surgery typically lasted 40 min and anaesthesia was reversed by injection of naloxone (0·1 mg kg^{-1} I.P.; Sigma). As animals began to recover from the anaesthetic they were given Vetergesic, the long-lasting narcotic partial agonist buprenorphine (0·1 mg kg^{-1} S.C.; Reckitt & Colman Pharmaceuticals, Hull, UK), for pain relief. Occasionally, when surgery took longer the animal was given a further injection of 0·1 ml Hypnorm I.M. every 10 min beyond 30 min.

Once surgical anaesthesia was established animals were placed in a stereotaxic frame. Body temperature was maintained at 37 °C throughout the surgery with an isothermal heating pad (Braintree Scientific, Braintree, MA, USA). In one group of rats a

* To whom correspondence should be addressed.

This manuscript was accepted as a Short Paper for rapid publication.

platinum–polymer poly(*O*-phenylenediamine–glucose oxidase (Pt–PPD–GOx) electrode was implanted in the right striatum. In another group a 2T platinum–iradium (Pt–Ir) electrode was implanted in the right striatum and a carbon paste electrode in the left striatum. The co-ordinates, with the skull levelled between bregma and lambda, were: anteroposterior +1·0 from bregma, mediolateral ± 2·5, and dorsoventral −8·5 from dura. The reference electrode (8T silver wire, 200 μm bare diameter; Advent Research Materials, Suffolk, UK) was placed in the cortex, the auxiliary electrode (8T silver wire) was placed between the skull and dura, and an earth wire (8T silver wire) was attached to one of the support screws (Oxford Seals & Bearings, Oxford, UK) which were used with dental acrylate (Associated Dental Products Ltd, Swindon, Wilts, UK) to fix the electrodes to the skull.

Animals were assessed for good health using the guidelines of Morton & Griffiths (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 Home Office Animals (Scientific Procedures) Act, 1986. At the end of the experiment animals were killed with an overdose of anaesthetic.

Working electrode preparation

Carbon paste electrodes were made, as described previously (Lowry, Boutelle, O'Neill & Fillenz, 1996*b*), from 5 cm lengths of Teflon-coated Pt–Ir (90%–10%) wire (125 μm bare diameter, 160 μm coated diameter (5T), Advent Research Materials). A 4 mm section of the insulation was cut from one end and a gold electrical contact (Semat Technical Ltd, Herts, UK) soldered to the bare wire. The Teflon was then slid along the wire to create an approximately 2 mm deep cavity which was packed with carbon paste using a bare Pt–Ir wire as plunger. When packed, the carbon paste surface was smoothed by tapping several times on a sheet of clean paper.

The hydrogen detection electrode was made from 2T (50 μm bare diameter, 75 μm coated diameter) Teflon-coated Pt–Ir (90%–10%) wire (Advent Research Materials) as described previously (Fellows & Boutelle, 1993).

Glucose sensors were based on the immobilization of glucose oxidase (GOx) on Pt electrodes with the polymer PPD (Lowry & O'Neill, 1994). GOx was immobilized on 5 cm lengths of 5T Teflon-coated Pt–Ir wire in PPD by potentiostatic electropolymerization of the *O*-phenylenediamine monomer (300 mmol l^{-1}) following a previously reported procedure (Lowry, McAteer, El Atrash, Duff & O'Neill, 1994).

Prior to implantation all Pt–PPD–GOx electrodes were calibrated *in vitro* for both glucose (0–100 mmol l^{-1}) and ascorbic acid (0–1 mmol l^{-1}) to ensure the selectivity of the sensor for glucose and linearity of response (Lowry & O'Neill, 1994). The *in vitro* calibration provides a reliable estimate for *in vivo* concentrations (J. P. Lowry, unpublished observations).

Voltammetric techniques *in vivo*

Constant potential amperometry (CPA) at +700 mV was used to oxidise H_2O_2 (the product of the glucose–GOx reaction) at the Pt–PPD–GOx electrodes *in vivo*. CPA was also used to detect hydrogen oxidation with the 2T Pt–Ir electrode being held at +250 mV. At this potential, detection of H_2 is mass-transport limited and is not compromized by detection of other oxidizable species present in the brain (Fellows & Boutelle, 1993). Changes in oxygen at implanted carbon paste electrodes were monitored using differential pulse amperometry (Lowry *et al.* 1996*b*). At the end of experiments, after removal from the brain, the carbon paste electrodes were calibrated for their oxygen sensitivity (Lowry, Boutelle & Fillenz, 1996*a*).

Regional cerebral blood flow measurements

In animals used in the blood flow measurements hydrogen was delivered to the animal's nose following a modified form of a previously reported procedure for use on freely moving animals (Fellows & Boutelle, 1993). Lightweight polyethyletherketone (PEEK) HPLC tubing (i.d., 1·6 mm; o.d., 3·2 mm; Anachem Ltd, Luton, Beds, UK) was held under the animal's snout at a distance of *ca* 5 mm. The PEEK tubing was connected through a flashback arrestor and a pressure regulator to a hydrogen cylinder (zero grade, British Oxygen Co., Guildford, Surrey, UK), with the gas flow controlled outside the rat's bowl. The gas was administered for approximately 5–10 s, typically at 5 min intervals and at a flow rate of 150 ml min^{-1}.

In a typical blood flow measurement the baseline was recorded for 5 s, hydrogen was then administered until the current (I) had increased by 15–30 nA. The decay curve data were collected for a 3–4 min period. Data points between 30 s and 4 min were analysed using a first order plot of $\ln(I_t - I_{baseline})$ *versus* time (where I_t and $I_{baseline}$ are current at time t and baseline current, respectively). If a linear plot was obtained, the slope was converted to blood flow (assuming a tissue partition coefficient of unity) (Fellows & Boutelle, 1993). Analysis was not performed if $I_{baseline}$ either side of the hydrogen transient differed by more than 1 nA.

Physiological stimulation

Tail pinch involved attaching a paper clip *ca* 3 cm from the tip of the rat's tail for 5 min; this produces a well characterized behaviour pattern which consists of gnawing, licking, eating and a general increase in the level of motor activity.

Results are given as means ± S.E.M. (n, number of observations).

RESULTS

Changes in rCBF and oxygen

Measurement of changes in rCBF was carried out at 5 min intervals. The mean basal rCBF was 86·5 ± 7·9 ml (100 g)$^{-1}$ min^{-1} ($n = 9$). Figure 1*A* shows the rCBF monitored over a period of 75 min at a platinum electrode implanted in the right striatum. During this period two tail pinches were administered by attaching a paper clip to the rat's tail. The 5 min tail pinch caused an increase in rCBF which was maximal in the first measurement after the onset of the tail pinch and returned to baseline level within the next one or two measurements after the end of the tail pinch. The mean increase in rCBF was 73·4 ± 8·4 ml (100 g)$^{-1}$ min^{-1} ($n = 9$), which is an increase of 88 ± 11% above basal.

Figure 1*B* shows the simultaneous measurement of tissue oxygen measured as the oxygen current at a carbon paste electrode implanted in the left striatum. The application of the tail pinch produced an immediate increase in oxygen current that reached a maximum at the end of the tail pinch and began to decrease as soon as the paper clip was removed. The mean baseline current was 194 ± 65 nA and the mean increase 10·4 ± 1·7 nA ($n = 9$). These figures

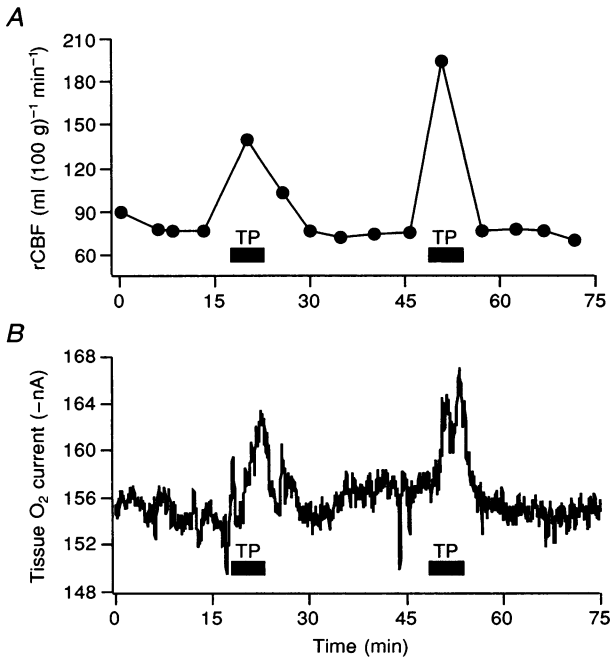

Figure 1. Simultaneous measurement of changes in regional cerebral blood flow (rCBF) and tissue oxygen in response to neuronal stimulation

A, meaurement of rCBF at a platinum electrode implanted in the right striatum. *B*, simultaneous measurement of oxygen current at a carbon paste electrode implanted in the left striatum. TP marks the application of a 5 min tail pinch.

cannot be used to calculate the percentage change, since the carbon paste electrode has a substantial background current. However using the *in vitro* calibration, carried out after the removal of the electrode, the increases in oxygen current were converted to oxygen concentration and gave a mean increase of $39 \pm 5{\cdot}5$ μM O_2; taking basal oxygen concentrations as 50 μM (Zimmerman & Wightman, 1991) this gives a percentage increase of 78 ± 11 % above basal.

The time for return to basal level of the oxygen current was $9{\cdot}8 \pm 3$ min; at no time was there a decrease below basal level.

Changes in glucose

In a separate group of rats changes in extracellular glucose were measured at a glucose oxidase electrode implanted in the right striatum. A typical example is shown in Fig. 2. In contrast to rCBF and tissue oxygen, the application of the tail pinch produced a decrease in the glucose current of $0{\cdot}86 \pm 0{\cdot}21$ nA from a mean basal value of $15{\cdot}3 \pm 2{\cdot}1$ nA ($n = 11$), which represents a decrease of $5{\cdot}2 \pm 0{\cdot}85$ %. On removal of the paper clip the glucose current began to rise and reached a peak of $1{\cdot}8 \pm 0{\cdot}3$ nA above basal 10 ± 3 min after the end of the tail pinch, which represents a peak increase of $11{\cdot}3 \pm 1{\cdot}1$ %. The time of return to basal glucose current was 30 ± 3 min, and occasionally glucose levels fell below basal. Using the *in vitro* calibration curve the changes in glucose can be converted to concentrations; this gives a mean decrease of $16{\cdot}2 \pm 2{\cdot}5$ μM and a mean increase of $34{\cdot}1 \pm 4{\cdot}3$ μM.

DISCUSSION

Tail pinch increases neuronal activation in the striatum as shown by the increased release of dopamine and ascorbate (Boutelle, Zetterstrom, Pei, Svensson & Fillenz, 1990). Coextensive with the increased neuronal activity there was

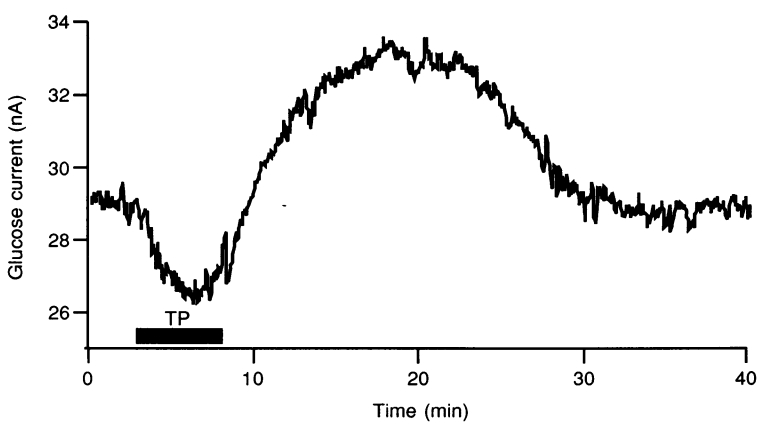

Figure 2. Changes in glucose in response to neuronal stimulation

Glucose current was recorded at a glucose oxidase electrode implanted in the right striatum. TP marks the application of a 5 min tail pinch.

an increase in rCBF. The hydrogen clearance measurements show that the increase in rCBF was rapid and began to decline as soon as motor activity ceased. The carbon paste electrode gives a continuous measure of tissue oxygen, which is a balance between supply and utilization. The close correspondence in the size and time course of the increase in rCBF and oxygen, measured as a percentage of basal, suggests that the changes in oxygen current are simply a reflection of changes in rCBF; there appears to be an increase in supply with no change in oxygen utilization. Since the oxygen current did not decrease below basal level at anytime, there appears to be no increased oxygen utilization either before or after the increase in rCBF.

Using microdialysis with a sampling time of 2·5 min, we have previously shown that tail pinch induces a brief decrease followed by an increase in glucose. Microdialysis provides an indirect measure of extracellular glucose which depends on the *in vivo* recovery. This cannot be derived from the *in vitro* probe recovery (Morrison, Bungay, Hsiao, Ball, Mefford & Dedrick, 1991). The glucose oxidase electrode provides a direct and continuous measure of extracellular glucose, which is the balance between supply and utilization. The 5% decrease in glucose during the tail pinch, which is very similar to the size of the decrease measured with microdialysis (Fray *et al.* 1996), must represent an increase in utilization, since it occurs during the period of increased rCBF, and hence presumably not a fall in glucose supply. Furthermore, the absence of an increase in oxygen utilization suggests neuronal aerobic glycolysis. During this period there is an increase in extracellular lactate, which partly overlaps the decrease in glucose; the dependence of this increase in lactate on glutamate uptake suggests that it is derived from astrocytes (Fray *et al.* 1996). In order to provide energy for neuronal metabolism, the lactate supplied by the astrocytes has to undergo oxidative phosphorylation; since there is no increase in oxygen consumption during neuronal activation, some ATP could be derived from neuronal glycolysis. It is not known whether lactate resulting from neuronal glycolysis passes into the extracellular fluid. Although there is much evidence that lactate is exported by astrocytes and utilized by neurones as a metabolic substrate (Tsacopoulos & Magistretti, 1996), some of the extracellular lactate in response to tail pinch could also be derived from neuronal glycolysis.

At the end of the tail pinch there was a slow rise in extracellular glucose which took 30 min to return to baseline level. The size of this increase as a percentage of basal is also very similar to that seen using microdialysis (Fray *et al.* 1996). The fact that the rise in glucose occurs when rCBF has returned to basal levels means that the rise in glucose cannot be derived directly from the bloodstream but must come from some other compartment. Evidence from a number of sources suggests that astrocytes constitute this compartment (Forsyth, Fray, Boutelle, Fillenz, Middleditch & Burchell, 1996; Tsacopoulos & Magistretti, 1996).

There remains the problem of the apparent absence of an increase in oxygen utilization. Since there is little or no transport of lactate from brain to blood, the lactate, whether derived from neurones or astrocytes, must ultimately undergo oxidative phosphorylation which requires increased oxygen utilization. At present there is no indication when that occurs.

Functional magnetic resonance imaging depends on the dissociation of rCBF and oxygen utilization in response to neuronal activation resulting in an increase in haemoglobin saturation. The present results suggest that this applies to activation of the striatum. However there have been reports that certain forms of stimulation in certain brain regions lead to a parallel increase in rCBF and rCM_{O_2} (Marrett *et al.* 1993); this would make it blind to functional magnetic resonance imaging.

In conclusion these preliminary experiments emphasize that continuous, simultaneous measurement of rCBF, oxygen and glucose provide an important approach to the elucidation of the problem of energy supply and utilization.

Baron, J. C., Lebrun-Grandie, P., Collard, P., Crouzel, C., Mestelan, G. & Bousser, M. G. (1982). Noninvasive measurement of blood flow, oxygen consumption, and glucose utilization in the same brain regions in man by positron emission tomography: Concise communication. *Journal of Nuclear Medicine* **23**, 391–399.

Boutelle, M. G., Zetterstrom, T., Pei, Q., Svensson, L. & Fillenz, M. (1990). *In vivo* neurochemical effects of tail pinch. *Journal of Neuroscience Methods* **34**, 151–157.

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.

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

Fox, P. T. & Raichle, M. E. (1986). Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. *Proceedings of the National Academy of Sciences of the USA* **83**, 1140–1144.

Fox, P. T., Raichle, M. E., Mintun, M. A. & Dence, C. (1988). Non-oxidative glucose consumption during focal physiologic neural activity. *Science* **241**, 462–464.

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., Boutelle, M. G. & Fillenz, M. (1996*a*). Measurement of brain tissue oxygen at a carbon paste electrode can serve as an index of increases in regional cerebral blood flow. *Journal of Neuroscience Methods* (in the Press).

LOWRY, J. P., BOUTELLE, M. G., O'NEILL, R. D., & FILLENZ, M. (1996*b*). Characterisation of carbon paste electrodes *in vitro* for simultaneous amperometric measurement of changes in oxygen and ascorbic acid concentrations *in vivo*. *Analyst* **121**, 761–766.

LOWRY, J. P., MCATEER, K., EL ATRASH, S. S., DUFF, A. & O'NEILL, R. D. (1994). Characterization of glucose oxidase-modified poly(phenylenediamine)-coated electrodes *in vitro* and *in vivo*: Homogeneous interference by ascorbic acid in hydrogen peroxide detection. *Analytical Chemistry* **66**, 1754–1761.

LOWRY, J. P. & O'NEILL, R. D. (1994). Partial characterization *in vitro* of glucose oxidase-modified poly(phenylenediamine)-coated electrodes for neurochemical analysis *in vivo*. *Electroanalysis* **6**, 369–379.

MARRETT, S., FUJITA, H., RIBEIRO, L., KUWABARA, H., MEYER, E., EVANS, A. C. & GJEDDE, A. (1993). Evidence for stimulus-specific changes in oxidative metabolism. *Annals of Nuclear Medicine* **7**, S120–121.

MORRISON, P. F., BUNGAY, P. M., HSIAO, J. K., BALL, B. A., MEFFORD, I. N. & DEDRICK, R. L. (1991). Quantitative microdyalisis: 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 a hypothesis for assessment. *Veterinary Record* **116**, 431–436.

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

TSACOPOULOS, M. & MAGISTRETTI, P. (1996). Metabolic coupling between glia and neurons. *Journal of Neuroscience* **16**, 877–885.

WOLFENSOHN, S. & LLOYD, M. (1994). *Handbook of Laboratory Animal Management and Welfare*. Oxford University Press, Oxford, UK.

ZIMMERMAN, J. B. & WIGHTMAN, R. M. (1991). Simultaneous electrochemical measurement of oxygen and dopamine *in vivo*. *Analytical Chemistry* **63**, 24–28.

Acknowledgements

J.P.L. gratefully acknowledges the European Commission for a research training fellowship under the Human Capital and Mobility programme (grant no. ERB CHB ICT94 1353). We thank Dr Martyn Boutelle for helpful discussions.

Author's email address

M. Fillenz: fillenz@vax.ox.ac.uk

Received 14 October 1996; accepted 22 November 1996.