The mechanisms controlling physiologically stimulated changes in rat brain glucose and lactate: a microdialysis study

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1. This study is concerned with the supply of metabolic substrates for neuronal metabolism. Experiments were carried out to investigate whether mechanisms demonstrated in cultured astrocytes also occurred *in vivo*; these were cAMP-mediated breakdown of glycogen and glutamate uptake-stimulated release of lactate.

2. *In vivo* microdialysis was used in freely moving rats. Lactate and glucose in the dialysate were assayed using enzyme-based on-line assays. Drugs were given locally through the dialysis probe. Regional cerebral blood flow was measured using the hydrogen clearance method.

3. There was an increase in dialysate glucose in response to the β-adrenoceptor agonist isoprenaline and to 8-bromo-cAMP, an analogue of cAMP, the second messenger of β-adrenoceptor stimulation. The effect of isoprenaline was blocked by the antagonist propranolol. Isoprenaline had no effect on dialysate lactate, which was increased by the glutamate uptake blocker β-D,L-threohydroxyaspartate (THA).

4. Physiological stimulation of neuronal activity produced an increase in both lactate and glucose. The increase in lactate was depressed in the presence of THA but was unaffected by propranolol. The increase in glucose was blocked by propranolol. Regional cerebral blood flow was increased by physiological stimulation but was unaffected by propranolol.

5. These results demonstrate that physiologically stimulated increases in glucose and lactate in the brain are mediated by different mechanisms.

Neurones show high rates of energy consumption, required for the maintenance of ionic gradients across their membranes by active transport mechanisms. They have, with a few exceptions, no energy stores. The generally accepted view is that their energy requirements, which fluctuate with the level of their activity, are met by variations in local cerebral blood flow and that this supplies glucose directly to the extracellular compartment (ECF) from which it enters neurones by facilitated diffusion.

A number of findings are not easily accommodated by this model. Among these is the finding in both human (Prichard *et al.* 1991) and animal experiments (Fellows, Boutelle & Fillenz, 1993) that an increase in neuronal activity is accompanied by a rapid rise in lactate in the presence of normal oxygen tensions. Since there is little or no transport of lactate across the blood–brain barrier (BBB) in adult organisms (Kuhr, van den Berg & Korf, 1988) this lactate must be derived from a source within the BBB. Using microdialysis we have also shown that physiological stimulation of neuronal activity is associated with a rise in dialysate glucose concentration, which has a very much longer duration than the parallel increase in regional cerebral blood flow (rCBF) (Fellows & Boutelle, 1993), suggesting that the changes in glucose concentration are not a simple consequence of increases in rCBF.

Astrocytes intervene between the blood vascular system and neurones since they have end-feet closely applied to the cerebral capillaries (Wolff, 1970) and processes which closely invest synaptic complexes (Derouiche & Frotscher, 1991), the brain region of maximum energy requirements. They have a store of glycogen (Cataldo & Broadwell, 1986) with a rapid turnover. Experiments *in vitro* with both cultured astrocytes and cortical slices have shown that glycogen breakdown is stimulated by a cAMP-dependent mechanism. In cortical slices, glycogen breakdown has been produced by the activation of β-adrenoceptors, which are positively coupled to adenylate cyclase (Magistretti, Sorg & Martin, 1993).

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The nature and fate of the products of astrocytic glycogenolysis are, at present, not clear. They could be utilized within the astrocyte for its own energy requirements or they could be exported as lactate or glucose. Cultured astrocytes transferred to glucose-free medium release pyruvate and lactate (Selak, Skaper & Varon, 1985; Dringen, Gebhardt & Hamprecht, 1993). Although glucose export from astrocytes is not demonstrable under such conditions (Forsyth, Bartlett & Eyre, 1996*a*), there is immunohistochemical evidence for the presence of glucose 6-phosphatase in astrocytes both *in vivo* (Bell, Hume, Busutill & Burchell, 1993) and *in vitro* (Forsyth, Bartlett, Burchell, Scott & Eyre, 1993) and presumptive evidence for the transporters which would be required for glucose export derived from glycogen (Forsyth *et al.* 1993). The dephosphorylation of non-tracer concentrations of (2-deoxy) glucose 6-phosphate and export of labelled (2-deoxy) glucose from astrocytes has been demonstrated *in vitro* (Forsyth *et al.* 1996*a*).

In previous reports we have shown that physiological stimulation of local neuronal activity in freely moving rats also results in rapid, reproducible increases in the concentration of lactate in striatal dialysate (Fellows *et al.* 1993). Such observations are consistent with NMR measurement of lactate (Prichard *et al.* 1991) and compatible with the disparity between glucose and O_2 consumption measured by positron emission tomography (Fox, Raichle, Mintun & Dence, 1988). Astrocytic release of lactate derived from glycogenolysis has been demonstrated *in vitro* (Dringen *et al.* 1993). This could provide a basis for the stimulated release of lactate observed *in vivo* although an alternative mechanism has recently been proposed. Glutamate uptake leads to lactate release in cultured astrocytes by stimulation of astrocytic glycolysis, a process that is mediated by the Na^+-dependent glutamate transporter (Pellerin & Magistretti, 1994). Similarly, in a preparation of acutely isolated photoreceptors and their associated Müller cells, there is an increased release of lactate from Müller cells in the dark (when photoreceptors are releasing neurotransmitter), which is taken up by the photoreceptors (Poitry-Yamate, Poitry & Tsacopoulos, 1995). The only neurotransmitter in this preparation is glutamate, released by the photoreceptors.

What has not been demonstrated so far is whether these mechanisms occur *in vivo*. The present study uses microdialysis in freely moving rats to assess whether the increases in glucose and lactate that occur upon physiological stimulation of local neuronal activity are mediated by β-receptor activation and/or glutamate uptake. We measured glucose and lactate in dialysate using an enzyme-based assay system in order to monitor changes resulting from both physiological stimulation and local infusion of drugs shown to alter astrocytic glycogenolysis *in vitro*. Although concentrations in the dialysate are not the same as extracellular concentrations, changes in the dialysate concentration reflect changes in flux throughout the ECF (Bungay, Morrison & Dedrick, 1990; Boutelle & Fillenz, 1996*a*). In parallel with these experiments, we have measured the effect of local administration of drugs on rCBF using a novel combined probe.

Some of this work has been submitted as a communication to The Physiological Society (Boutelle & Fillenz, 1996*b*).

METHODS

Microdialysis probe construction

Concentric microdialysis probes were constructed by inserting a plastic-coated silica tube (Scientific Glass Engineering, Milton Keynes, UK) and a tungsten wire (Goodfellow Metals, Cambridge, UK) into a polyacrylonitrile dialysis fibre (Hospal Industrie, Meyzieu, France) and gluing the fibre into a 12 mm stainless steel cannula (Goodfellow Metals). The tip was sealed with epoxy glue and a second silica tube inserted into the cannula to form the outlet. The total exposed fibre length was between 3·8 and 4·2 mm and the external diameter of the sampling region of the probe was 300 μm.

Combined microdialysis regional cerebral blood flow probe

In some experiments we wished to examine the effects of local drug infusion on rCBF. We have previously described an adaptation of the hydrogen clearance method to allow the measurement of rCBF in the freely moving rat (Fellows & Boutelle, 1993). This uses an implanted platinum wire electrode as an amperometric detector for tissue hydrogen levels. For the present study we have developed a new implanted device which combines a microdialysis probe (for local drug infusion) with a hydrogen detection electrode. A short length (4·5 mm) of insulation was stripped from each end of a 40 mm length of Teflon-coated platinum/10% iridium wire (o.d., 75 μm; wire diameter, 50 μm; Advent Research Materials, Halesworth, UK). Under a dissecting microscope, a length (~250 μm) of exposed wire was bent through a 90 deg angle to provide an anchor and hooked around the tip of a microdialysis probe such that the remainder of the exposed wire was laid along the probe membrane. Epoxy glue (Devcon Ltd, Shannon, Ireland) was then used to attach the wire to the steel shaft of the microdialysis probe, and the anchor to the probe tip. Care was taken to ensure that the platinum wire lay close to the probe membrane, and that there was no electrical contact between the platinum wire and steel shaft or the internal tungsten wire of the probe.

Surgical procedures

Male Sprague–Dawley rats weighing 200–300 g were anaesthetized, following published guidelines (Wolfensohn & Lloyd, 1994), with the following mixture: (a) Hypnorm (Janssen Pharmaceuticals Ltd, Oxford, UK), a combination of the neuroleptic analgesic fentanyl (0·318 mg ml^{-1}) and fluanisone (10 mg ml^{-1}), and (b) Hypnovel (Roche), the benzodiazepine midazolam (5 mg ml^{-1}), and (c) sterile water, mixed in the ratio 1:1:2. This mixture was injected I.P. at a volume of 3·3 ml (kg body weight)$^{-1}$, 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 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, the long-lasting narcotic partial agonist buprenorphine (0·1 mg kg^{-1} S.C.; Reckitt and Colman Pharmaceuticals, Hull, UK), for pain relief. Occasionally, when surgery took longer the animal was given a further I.M. injection of 0·1 ml of Hypnorm every 10 min beyond the initial 30 min.

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 anterior–posterior from bregma, 2·5 mm median–lateral and −8·5 mm below dura (Paxinos & Watson, 1986). Probes were secured in position using dental screws and acrylic (Associated Dental Products Ltd, Swindon, UK). Where measurement of rCBF was required, the combined microdialysis–rCBF probe was implanted using the same procedure. In addition, as described elsewhere (Fellows & Boutelle, 1993), auxiliary and earth electrodes, made of silver wire, were placed between the skull and the dura using the same hole as used for the probe. A chloridized silver reference electrode was placed in the contralateral cortex. All wires were Teflon-coated silver with the insulation stripped to give 2 mm exposed wire (o.d., 270 μm; wire diameter, 200 μm; Advent Research Materials).

Animals were allowed 24 h recovery. Animals 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, 1985); 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. 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.

Experimental procedures

After surgery, rats were kept in large plastic bowls (diameter, ~50 cm) and allowed free access to food and water. Experiments were carried out in the same 'home bowl' 24 h after surgery. At the start of each experiment, probes were perfused with artificial cerebrospinal fluid (ACSF; 147 mM NaCl, 4 mM KCl, 1·2 mM $CaCl_2$ and 1 mM $MgCl_2$; see de Boer, Damsma, Fibiger, Timmerman, deVries & Westerink, 1990) at 2 μl min^{-1} using a CMA 100 microdialysis pump. The volume collected was checked to ensure that the probe was not leaking. The dialysate was then passed through fluoropolymer tubing (Biotech Instruments Ltd, Luton, UK) and through a pre-oxidation system of tubular electrodes in order to remove ascorbic acid and other electrochemical interferents (Berners, Boutelle & Fillenz, 1994) and the volume checked again. Sequential 5 μl volumes of dialysate were automatically injected into the assay system described below using a CMA/160 on-line injector (CMA Microdialysis, Stockholm, Sweden). Drugs were added to the perfusion fluid for the appropriate time intervals.

Glucose and lactate assay

Dialysate in 5 μl aliquots was assayed for glucose or lactate using ferrocene-mediated enzymatic detection. The preparation of the enzyme-packed bed used in this system is described elsewhere (Boutelle, Fellows & Cook, 1992). Glucose or lactate oxidase (Genzyme Diagnostics, West Malling, UK) and horseradish peroxidase (Boehringer Mannheim) were immobilized on 10 μM tresyl chloride-activated silica beads and slurry packed into a guard column (Anachem Ltd, Luton, UK). The beds remained active for 3–9 months.

The enzyme bed was perfused with ferrocene buffer (1·5 mM ferrocene monocarboxylate, 100 mM Na_2HPO_4, 1 mM EDTA and 0·05% Kathon CG) at 0·3 ml min^{-1} using an HPLC pump. Glucose was oxidized to gluconolactone and H_2O_2 and lactate was oxidized to pyruvate and H_2O_2. The remaining reaction scheme was identical for glucose and lactate. H_2O_2 was oxidized by horseradish peroxidase to produce water and two ferricinium species in the buffer. This was then reduced at a downstream glassy carbon electrode to give a single current peak. The detection electrode was held at 0 mV with respect to a Ag–AgCl reference electrode by a potentiostat (Electrochemical and Medical Systems Ltd, Newbury, UK), which was connected to a chart recorder.

The bed was calibrated at the beginning and end of each experimental run using a four-point calibration from 50 to 500 μM in the case of glucose, and a final calibration of standards covering the range within each experiment for lactate (typically 25–200 μM). Glucose standards were made fresh every day from mutorotated stock, made 24 h before each experiment using D-glucose (BDH) and ACSF. Lactate standards were made from fresh sodium lactate stock.

Regional cerebral blood flow measurements

In animals used in the blood flow measurements hydrogen was delivered to the animal's nose in one of two ways. (1) A lightweight polyethyletherketone (PEEK) HPLC tube (i.d., 1·6 mm; o.d., 3·2 mm; Anachem) was passed through channels in the dental acrylate, and bent in such a way as to position two 0·4 mm holes about 5 mm beneath the rat's nostrils (Fellows & Boutelle, 1993) This lightweight, rigid structure was well tolerated by the rats, which exhibited normal behaviour while it was in place. (2) A PEEK tube was held near the animal's nose. In either case, the PEEK tubing was connected through a flashback arrestor and a pressure regulator to a hydrogen cylinder (zero grade, British Oxygen Co., Guildford, UK), with the gas flow controlled outside the rat's bowl. Measurements could be made without disturbing the animal or interrupting periods of behaviour or sleep. The gas was administered for approximately 5–10 s, typically at 5 min intervals and at a flow rate of 150 ml min^{-1}.

Hydrogen detection and data acquisition

The platinum detection electrode was held at 250 mV *versus* the implanted Ag–AgCl reference electrode with a low noise potentiostat (Electrochemical and Medical Systems Ltd). At this potential, detection of hydrogen is mass transport limited and is not compromised by detection of other oxidizable species present in the brain (Young, 1980). The current was digitized using a NB-MIO-16× multifunction board (National Instruments Corp., Austin, TX, USA) in a Macintosh Quadra 840av computer, and was controlled using LabVIEW software (National Instruments). Current values were sampled at 2 ms and averaged by the computer at 0·5 s intervals.

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 was collected for a 3–4 min period. Data points between 30 s and 4 min were analysed using a first-order plot of $\ln[i(t) - i(\text{baseline})]$ *versus* time t. If a linear plot was obtained, the slope was converted to blood flow (assuming a tissue partition coefficient of unity) according to the following formula:

$$\text{rCBF} = \text{slope} \times 6000,$$

where rCBF is expressed in ml (100 g tissue weight)$^{-1}$ min^{-1} and slope is measured in s^{-1}. Analysis was not performed if i(baseline) either side of the hydrogen transient differed by more than 1 nA.

Drugs

(±)-Isoprenaline hydrochloride and DL-propranolol hydrochloride were dissolved in ACSF immediately prior to *in vivo* infusion. 8-Bromo-cAMP (8-Br-cAMP; sodium salt) was dissolved to give a concentration of 1 mM. Aliquots (1 ml) were frozen, thawed and diluted down to 50 μM as required. β-D,L-Threohydroxyaspartate

(THA) was made up in ACSF and added prior to *in vivo* perfusion. All chemicals were purchased from Sigma unless otherwise stated.

Statistics

Changes are shown as a percentage of the mean baseline value calculated from the last six samples before the addition of a drug or the application of a stimulus. Statistical significance is calculated from absolute values by Student's paired t test. With physiological stimulation the efflux of lactate or glucose is expressed in absolute units and is calculated from the area under the curve using those samples which are different from baseline. Comparisons are made using the Mann–Whitney U test. Results are given as means ± S.E.M.

RESULTS

Basal levels of lactate and glucose

Basal lactate dialysate levels were 88 ± 6 μM ($n = 35$) and basal glucose dialysate levels were 195 ± 9 μM ($n = 53$). Both levels remained constant over periods of several hours, provided the animal remained quiescent. Spontaneous movements or grooming caused transient increases in the baseline, the lactate level being much more sensitive to such changes in activity than the glucose level. Local administration of drugs by addition to the perfusion medium, which involves disturbance of the animal and disconnection of the tubing, produces an injection artefact which precedes the arrival of the drug in the brain. This again tends to be more marked for lactate than glucose.

Effect of drugs

There is evidence from *in vitro* experiments that β-adrenoceptor stimulation causes a cAMP-mediated breakdown of glycogen. The striatum has a sparse noradrenergic innervation but a high density of β-adrenoceptors. We therefore examined the effect of local administration of the non-selective β-adrenoceptor agonist isoprenaline. A 5 min pulse of 50 μM isoprenaline was added to the perfusion medium. In experiments measuring either lactate or glucose this caused an immediate steep decrease in baseline during the period of drug infusion.

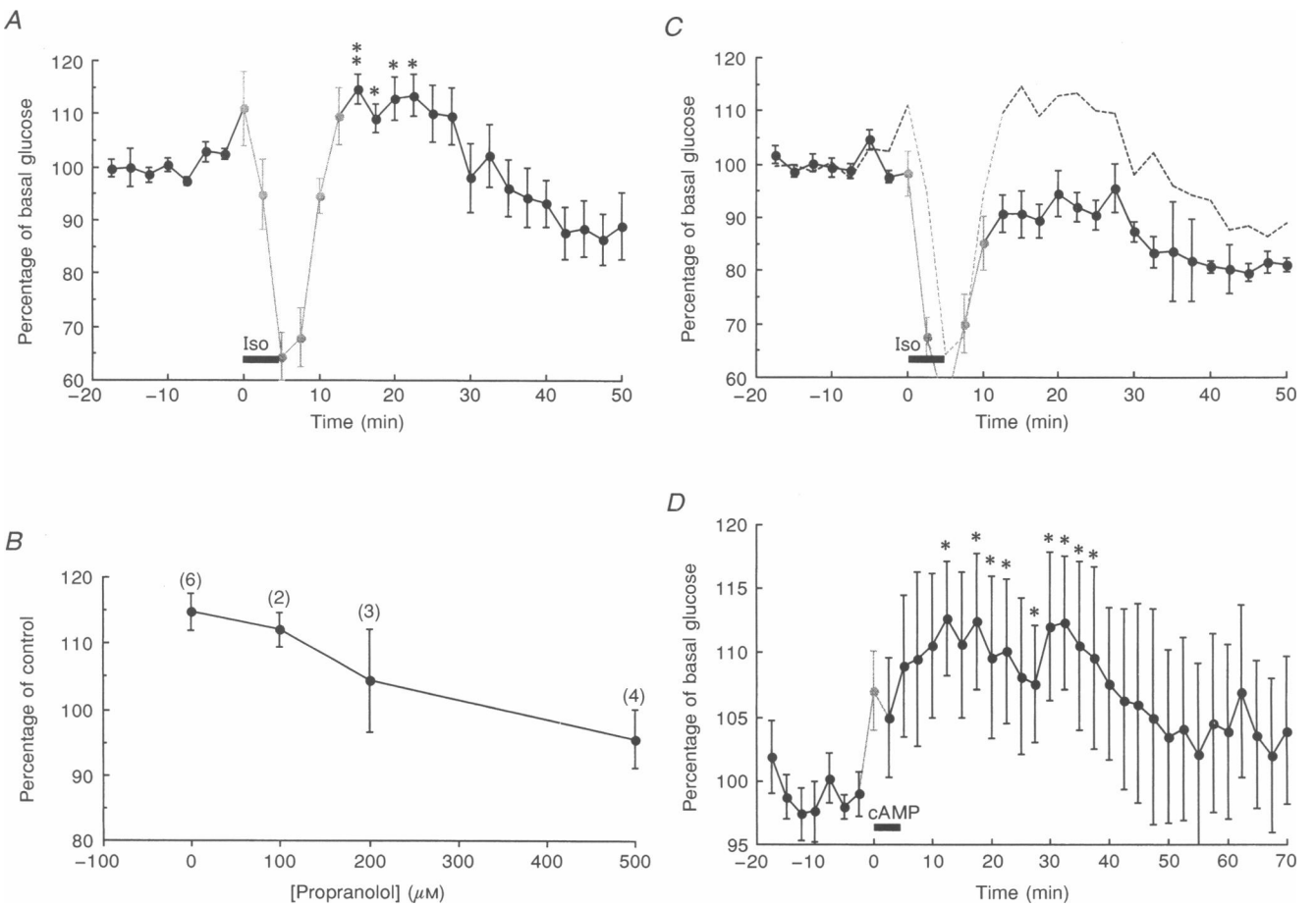

Figure 1. Effect of local drug infusion on dialysate glucose levels

Grey lines represent injection artefacts as discussed in the text. *A*, the effect of a 5 min infusion of 50 μM isoprenaline (Iso). *B*, dose–response curve for the effect of propranolol on the maximum isoprenaline-induced increase in glucose. Numbers in parentheses indicate *n*. *C*, the effect of a 5 min infusion of 50 μM isoprenaline in the presence of 500 μM propranolol in the perfusion medium. The dashed line shows the data from *A* for comparison. *D*, the effect of a 5 min infusion of 50 μM 8-Br-cAMP on dialysate glucose ($n = 4$). * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$ compared with control.

Since isoprenaline is an easily oxidized compound we tested its effect on the enzyme bed disconnected from the animal. The same decrease was seen when isoprenaline was injected into the enzyme bed together with either glucose or lactate. This suggests that the isoprenaline is being oxidized by the H_2O_2 intermediate in the enzyme bed. We therefore regarded the decrease in the *in vivo* traces as artefactual. In presenting the data we have shown the complete time course; the data points representing the above artefacts are shown in grey.

A 5 min pulse of 50 μM isoprenaline produced no change in the basal level of lactate beyond the 10 min period of the artefact.

When changes in glucose were measured in response to the addition of isoprenaline, there was an increase, which was maximal 20 min after the addition of the drug (Fig. 1*A*). There was then a slow decline to below baseline level. Any early change during the artefact was lost.

In order to confirm that the effect of isoprenaline was receptor mediated, we infused the β-adrenoceptor antagonist propranolol. Continuous infusion of propranolol alone (50 μM) produced a statistically significant decrease in basal glucose dialysate levels to $83 \pm 5\%$ of control ($P < 0{\cdot}05$; $n = 5$). With propranolol in the perfusion medium, there was a dose-dependent decrease in the isoprenaline-induced rise in glucose (Fig. 1*B*). Neither the artefact nor the reduction in basal glucose showed any dose dependency. When 500 μM propranolol was added to the perfusion medium, followed 20 min later by the 5 min pulse of 50 μM isoprenaline, there was no effect on the isoprenaline artefact but the increase in glucose was abolished (Fig. 1*C*).

As β-adrenoceptors stimulate adenylate cyclase we examined the effect on glucose of adding a 5 min pulse of 50 μM 8-Br-cAMP, a membrane-permeant analogue of cAMP. This caused an immediate increase in glucose (Fig. 1*D*). The increase is similar in size to that produced by isoprenaline, but longer in duration.

Since β-adrenoceptor stimulation did not appear to stimulate lactate export we examined the role of glutamate uptake. Experiments *in vitro* with astrocytes have shown that lactate release is produced by the Na^+-dependent uptake of glutamate and is inhibited by the uptake blocker THA (1 mM) (Pellerin & Magistretti, 1994). We added THA to the perfusion medium and measured the dialysate lactate. Concentrations between 1 and 100 μM had no effect; 1 mM THA produced a rapid increase to 300% of control levels (Fig. 2*A*).

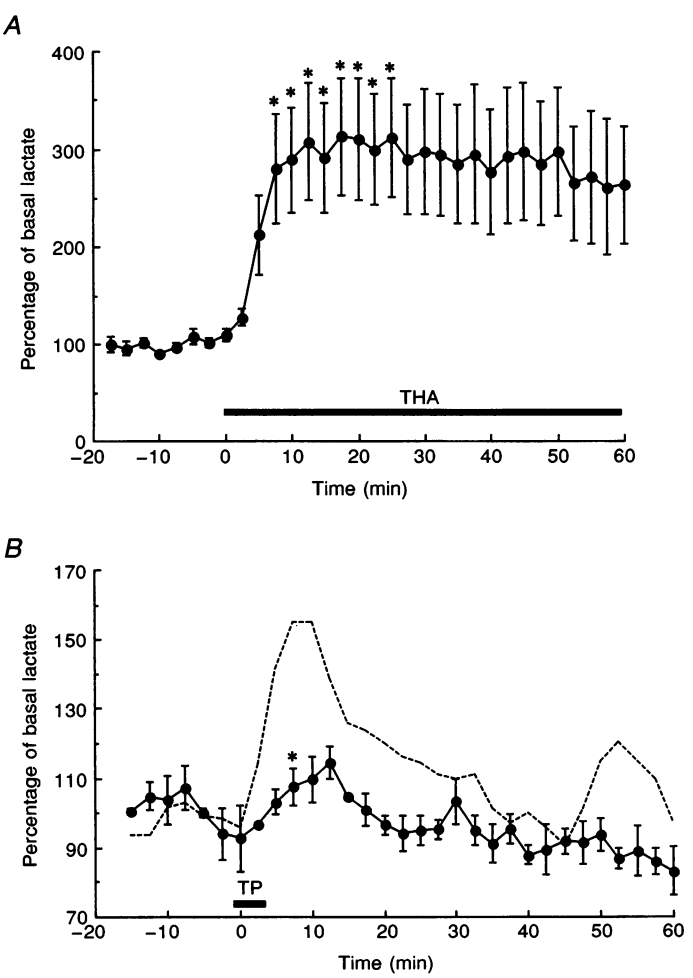

Figure 2. The effect of local infusion of the glutamate uptake blocker THA (1 mM) on dialysate lactate levels

A, effect of infusion on basal levels. *B*, a 5 min tail pinch (TP) in the presence of THA. The dashed line shows the data from Fig. 3*A* for comparison.
* $P < 0{\cdot}05$ compared with control.

Table 1. Regional blood flow measurements (rBCF) with no perfusion, perfusion with ACSF and perfusion with propranolol

	rCBF (ml (100 g)$^{-1}$ min^{-1})			
	Basal	n	Stimulated	n
No perfusion	76·0 ± 3·4	11	127·6 ± 6·7	7
Perfusion with ACSF	81·2 ± 4·6	8	124·3 ± 6·1	7
Perfusion with propranolol	74·2 ± 3·2	6	125·6 ± 5·6	7

Measurement of regional cerebral blood flow

In order to see whether the effects on glucose could be explained by effects of β-adrenoceptors on blood vessels we needed to study the effect of local drug administration on regional cerebral blood flow.

We have previously refined the hydrogen clearance method to allow rapid measurements of absolute rCBF in the freely moving animal. For this study we have developed a new combined probe which allows the measurement of rCBF in response to local application of drugs. Table 1 shows that the measured rCBF is unaffected by perfusion of ACSF through the probe. The addition of propranolol (50 μM) had no effect on basal rCBF.

Stimulated changes in lactate and glucose

We have shown previously that physiological stimulation leads to an increase in both lactate and glucose as well as rCBF (Fellows & Boutelle, 1993; Fellows *et al.* 1993). In order to discover whether these increases are mediated by the same mechanism, we examined the effect of drugs on these changes. We used either a 5 min tail pinch or stimulated grooming as the physiological stimulus.

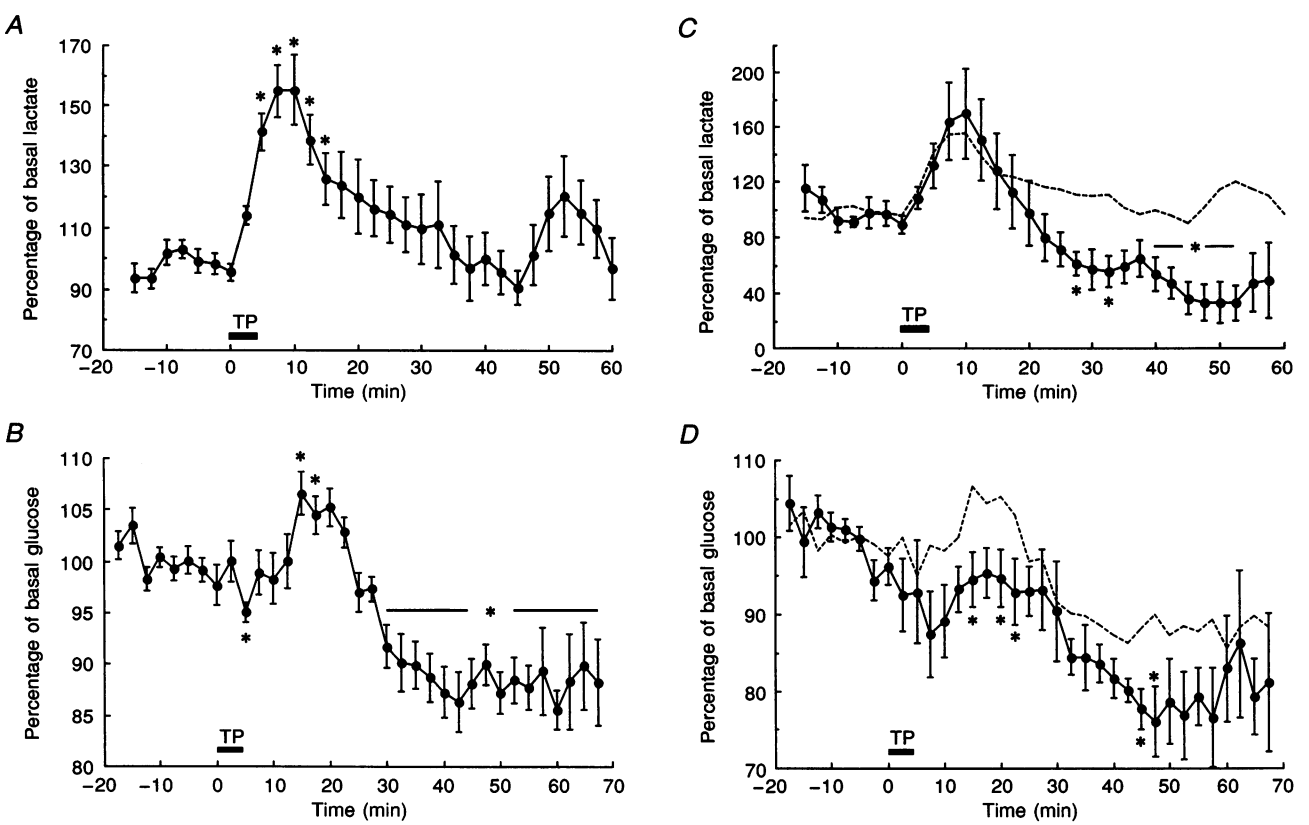

Figure 3. The effect of a 5 min tail pinch (TP) on dialysate lactate levels

A, lactate levels during perfusion with ACSF. *B*, glucose levels during perfusion with ACSF. *C*, lactate levels during perfusion with 50 μM propranolol. The dashed line shows the data from *A* for comparison. *D*, glucose levels during perfusion with 50 μM propranolol. The dashed line shows the data from *B* for comparison. * $P < 0·05$ compared with control.

With ACSF as the perfusion fluid, a 5 min tail pinch resulted in a brisk efflux of lactate as shown in Fig. 3*A*. The increase in lactate expressed in absolute units was 0·991 ± 0·28 nmoles ($n = 7$). The changes in glucose were more complex. There was a decrease in glucose at the end of the tail pinch, followed by a rise which reached a peak 12·5 min after the start of the tail pinch and was followed after 27·5 min by a decrease below baseline. The increase in glucose expressed in absolute units was 0·31 ± 0·1 nmoles ($n = 10$) (Fig. 3*B*).

When 1 mM THA was added to the perfusion medium there was a very large increase in the basal level of lactate (see above); the application of a tail pinch now produced a very much reduced response. Because there was great variability in the baseline increase with THA, the tail pinch response was normalized and shown as a percentage of baseline (Fig. 2*B*). Statistically significant changes from baseline are calculated using the absolute data. As can be seen, only one point following tail pinch is significantly different from baseline.

We next examined the role of β-adrenoceptors in the stimulus-evoked increase in glucose and lactate. Propranolol (50 μM) was added to the perfusion medium. In the presence of propranolol, the tail pinch-induced increase was unchanged but was followed by a decrease below baseline levels (Fig. 3*C*).

We carried out the same experiment and measured the changes in glucose. When the decrease in baseline produced by propranolol had reached a stable level (typically 30 min), the animal was given a 5 min tail pinch. There was still an early decrease in glucose, but the rise was abolished; the delayed decrease was enhanced (Fig. 3*D*).

Using the combined probe for measurement of rCBF we found that stimulated grooming increased rCBF to 127·6 ± 6·7 ml (100 g)$^{-1}$ min^{-1} ($P < 0·001$, $n = 7$) with a rapid return to basal at the end of the stimulation. Like the basal rCBF it was unaffected by perfusion of ACSF. This validates the measurement of rCBF during perfusion with the combined probe. Infusion of propranolol (50 μM) was followed 50 min later by stimulated grooming. Stimulated rCBF was 125·6 ± 5·6 ml (100 g)$^{-1}$ min^{-1} ($P < 0·001$, $n = 7$) (this was not significantly different from that in the absence of propranolol (see Table 1).

DISCUSSION

There is now a wealth of evidence to show that physiological stimulation such as tail pinch or induced grooming stimulates neuronal activity in the rat striatum (Pei, Zetterström & Fillenz, 1990; Wheeler, Boutelle & Fillenz, 1995). Such neuronal activity leads to an increase in energy consumption required for the restoration of ionic equilibria. The present experiments confirm our earlier findings that physiologically stimulated neuronal activity is accompanied by an increase in dialysate lactate (Fellows *et al.* 1993), glucose and rCBF (Fellows & Boutelle, 1993). The present experiments were designed to see whether the same or separate mechanisms were responsible for these changes.

Tail pinch causes a doubling of the dialysate lactate concentration and rCBF, although the lactate increase is much longer lasting. This increase in lactate is not due to lack of oxygen, since neuronal activation causes an increase in brain oxygen levels (Fox *et al.* 1988; Ogawa *et al.* 1992) and is unlikely to be derived from the blood. Although there is evidence for some transport of lactate across the BBB (Lear & Kasliwal, 1991), a brief and mild stimulus, such as tail pinch, is not likely to raise plasma lactate. Furthermore, Kuhr *et al.* (1988) found no significant contribution of plasma lactate to dialysate lactate under both basal and stimulated conditions. Recent evidence on the distribution of two isoenzymes of lactate dehydrogenase shows that neurones contain the isoenzyme that favours the formation of pyruvate from lactate whereas astrocytes contain the isoenzyme that preferentially catalyses the formation of lactate from pyruvate (Bittar, Carnay, Kiraly, Pellerin, Bouras & Magistretti, 1995) Also, in an elegant study, Tsacopoulos and colleagues (Tsacopoulos & Magistretti, 1996), using a complex of mammalian photoreceptors and their associated Müller glial cells, have shown that glial cells synthesize and preferentially release lactate and that even in the presence of glucose, lactate released from glial cells fuels the oxidative metabolism of photoreceptors (Poitry-Yamate *et al.* 1995). The group of Magistretti *et al.* has shown that in cultured astrocytes release of lactate is triggered by the uptake of glutamate (Pellerin & Magistretti, 1994). Receptor-mediated glycogenolysis may be an alternative source of astrocyte-derived lactate (Dringen *et al.* 1993). In the present experiments, isoprenaline, which stimulates adenylate cyclase and has been shown to lead to glycogenolysis *in vitro* (Magistretti *et al.* 1993), does not lead to an observable increase in lactate *in vivo*. A brief increase during the artefact would be lost, but if it is a cAMP-mediated effect, like the one responsible for the glucose increase, one would expect a similar time course. The strongest evidence against the involvement of β-receptors in the lactate increase is the finding that the physiologically stimulated increase in lactate is unaffected by propranolol. Propranolol does however decrease the lactate baseline and the level following the stimulated increase; this may be secondary to the decrease in glucose which may result in a greater uptake of lactate.

THA, which is a competitive inhibitor of glutamate uptake, produces a large increase in basal lactate. This could be due to the fact that it is itself transported by the carrier. In addition, the hydroxyaspartate isomers, of which THA is one, have been shown to be competitive inhibitors of the transamination enzyme aspartate amino transferase (Jenkins, 1961); this effect results in a reduction of oxidative metabolism and thus an accumulation of lactate.

Physiological stimulation added on top of the effect of THA produces no statistically significant increase. There could be two possible explanations for this. Glutamate uptake may be saturated by the high concentration of THA and physiologically released glutamate therefore has no further effect. Alternatively, lactate production may already be maximal and therefore not capable of further stimulation.

The physiologically stimulated change in glucose has a complex pattern and represents the balance between utilization and supply. The early and late decreases suggest that at these stages utilization exceeds supply. The increase in glucose is very much smaller than that of lactate and rCBF; furthermore, its duration is very much longer than the increase in rCBF, in agreement with our previous findings (Fellows & Boutelle, 1993). This implies that increases in ECF glucose concentration are not the direct result of local increases in rCBF.

Astrocytes comprise a possible additional source for glucose delivery to neurones. They have a small store of glycogen with a rapid turnover rate. This is broken down by a cAMP-mediated mechanism triggered by receptors positively coupled to adenylate cyclase, among which are β-adrenoceptors (Magistretti *et al.* 1993). In the present experiments the β-adrenoceptor agonist isoprenaline produced an increase in glucose which was blocked by propranolol and mimicked by the administration of 8-Br-cAMP.

Propranolol blocked the physiologically stimulated increase in glucose but had no effect on either stimulated increase in lactate or rCBF, again suggesting that glucose concentrations are not directly affected by local changes in rCBF. The recent finding that infusion of adrenaline does not increase rCBF (Horinaka, Artz, Jehle, Kennedy & Sokoloff, 1995) lends further support to the view that β-adrenoceptor-stimulated increase in glucose is not mediated by an increase in rCBF.

Although at present the evidence for export of glucose from astrocytes *in vitro* is equivocal (Forsyth *et al.* 1996*a*), the results of the present study suggest that it may occur *in vivo*. Astrocytes appear to express all elements of the multi-component glucose 6-phosphatase complex (Forsyth *et al.* 1993). Possible reasons why the *in vitro* environment may not favour glucose export have been discussed (Forsyth, 1996). The role of astrocytes in the provision of glucose for neuronal metabolism is reviewed elsewhere (Forsyth, Fray, Boutelle, Fillenz, Middleditch & Burchell, 1996*b*).

In vitro experiments (Tsacopoulos & Magistretti, 1996) show that glucose is taken up into astrocytes rather than neurones, is partly converted to glycogen and gives rise to lactate, which is exported in response to stimulation of photoreceptors. The stimulus for this export consists of the uptake of glutamate. Experiments with whole animals using $^{1}H/^{13}C$ NMR showed that the stimulated increase in brain lactate is derived from uptake of glucose not lactate from the blood (Petroff *et al.* 1992). In our microdialysis experiments we are measuring the flux of glucose and lactate through the ECF to neurones. Our blood flow experiments demonstrate that the uptake from the blood and the delivery to the ECF are sequential.

In conclusion the present experiments suggest that stimulated delivery of lactate and glucose from astrocytes to the ECF are mediated by different mechanisms. The stimulus is provided by changes in neuronal activity and the resulting transmitter release.

Bell, J. E., Hume, R., Busutill, A. & Burchell, A. (1993). Immunocytochemical detection of the microsomal glucose-6-phosphatase in human astrocytes. *Neuropathology and Applied Neurobiology* **19**, 429–435.

Berners, M. O. M., Boutelle, M. G. & Fillenz, M. (1994). On-line measurement of brain glutamate with an enzyme/polymer-coated tubular electrode. *Analytical Chemistry* **66**, 2017–2021.

Bittar, P., Carnay, Y., Kiraly, M., Pellerin, L., Bouras, C. & Magistretti, P. J. (1995). Selective distribution of lactate dehydrogenase in neurons and astrocytes: evidence for activity-dependent metabolic trafficking. *Neuroscience Abstracts* **21**, 656.1.

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*a*). Clinical microdialysis: The role of on-line measurement and quantitative microdialysis. *Acta Neurochirurgica* (in the Press).

Boutelle, M. G. & Fillenz, M. (1996*b*). A combined probe to study the pharmacology of physiologically stimulated retinal blood flow in the rat brain. *Journal of Physiology* **491.P**, 29*P*.

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

Cataldo, A. M. & Broadwell, R. D. (1986). Cytochemical identification of cerebral glycogen and glucose-6-phosphate activity under normal and experimental conditions. *Journal of Electron Microscopy Technique* **3**, 413–437.

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.

Derouiche, A. & Frotscher, M. (1991). Astroglial processes around identified glutamatergic synapses contain glutamine synthetase: evidence for transmitter degradation. *Brain Research* **552**, 346–350.

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. (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. (1996). Astrocytes and the delivery of glucose from plasma to neurons. *Neurochemistry International* **28**, 231–241.

Forsyth, R. J., Bartlett, K., Burchell, A., Scott, H. M. & Eyre, J. A. (1993). Astrocytic glucose-6-phosphatase and the permeability of brain microsomes to glucose-6-phosphate. *Biochemical Journal* **294**, 145–151.

Forsyth, R. J., Bartlett, K. & Eyre, J. (1996*a*). Dephosphorylation of 2-deoxyglucose 6-phosphate and 2-deoxy glucose export by cultured astrocytes. *Neurochemistry International* **28**, 243–250.

Forsyth, R. J., Fray, A., Boutelle, M. G., Fillenz, M., Middleditch, C. & Burchell, A. (1996*b*). A role for astrocytes in glucose delivery to neurons? *Developmental Neuroscience* suppl. **67**, 13–20.

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.

Horinaka, N., Artz, N., Jehle, J., Kennedy, C. & Sokoloff, L. (1995). Effect of continuous epinephrine infusion on cerebral blood flow. *Neuroscience Abstracts* **21**, 68.2.

Jenkins, W. T. (1961). Glutamic-aspartic transaminase VI. The reaction with certain beta-substituted aspartic acid analogues. *Journal of Biological Chemistry* **236**, 1121–1125.

Kuhr, W. G., van den Berg, C. J. & Korf, J. (1988). *In vivo* identification and quantitative evaluation of carrier-mediated transport of lactate at the cellular level in the striatum of conscious, freely moving rats. *Journal of Cerebral Blood Flow and Metabolism* **8**, 848–856.

Lear, J. L. & Kasliwal, R. (1991). Autoradiographic measurement of cerebral lactate transport rate constants in normal and activated conditions. *Journal of Cerebral Blood Flow and Metabolism* **11**, 576–580.

Magistretti, P. J., Sorg, O. & Martin, J.-L. (1993). Regulation of glycogen metabolism in astrocytes: physiological, pharmacological, and pathological aspects. In *Astrocytes*, ed. Murphy, S., pp. 243–265. Academic Press, San Diego, CA, USA.

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. *The Veterinary Record* **116**, 431–436.

Ogawa, S., Tank, D. W., Menon, R., Ellerman, J. M., Kim, S.-G., Merkle, H. & Ugurbil, K. (1992). Intrinsic signal changes accompanying sensory stimulation: Functional brain mapping with magnetic resonance imaging. *Proceedings of the National Academy of Sciences of the USA* **89**, 5951–5955.

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

Pei, Q., Zetterström, T. & Fillenz, M. (1990). Tail pinch-induced changes in the turnover and release of dopamine and 5-hydroxytryptamine in different brain regions of the rat. *Neuroscience* **35**, 133–138.

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.

Petroff, O. A., Novotny, E. J., Avison, M., Rothman, D. L., Alger, J. R., Ogino, T., Shulman, G. I. & Prichard, J. W. (1992). Cerebral lactate turnover after electroshock: *in vivo* measurements by $^{1}H/^{13}C$ magnetic resonance spectroscopy. *Journal of Cerebral Blood Flow and Metabolism* **12**, 1022–1029.

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.

Prichard, J., Rothman, D., Novotny, E., Petroff, O., Kuwaraba, T., Avison, M., Howseman, A., Hanstock, C. & Sulman, R. (1991). Lactate rise detected by ^{1}H NMR in human visual cortex during physiologic stimulation. *Proceedings of the National Academy of Sciences of the USA* **88**, 5829–5831.

Selak, I., Skaper, S. & Varon, S. (1985). Pyruvate participation in the low molecular weight trophic activity for CNS neurons in glia-conditioned medium. *Journal of Neuroscience* **5**, 23–28.

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

Wheeler, D., Boutelle, M. G. & Fillenz, M. (1995). The role of *N*-methyl-D-aspartate receptors in the regulation of physiologically released dopamine. *Neuroscience* **65**, 767–774.

Wolfensohn, S. & Lloyd, M. (1994). *Handbook of Laboratory Animal Management and Welfare*. Oxford University Press, Oxford.

Wolff, J. R. (1970). The astrocyte as link between capillary and nerve cell. *Triangle* **9**, 153–164.

Young, W. (1980). Hydrogen clearance measurement of blood flow: a review of techniques and polarographic principles. *Stroke* **11**, 552–564.

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