

BRES 18550

Rapid changes in extracellular glucose levels and blood flow in the striatum of the freely moving rat

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(Accepted 15 September 1992)

Key words: Microdialysis; Glucose; Cerebral blood flow; Hydrogen clearance; Unanaesthetised; Rat brain; Tail pinch; Restraint stress

The dynamics of regional cerebral blood flow and brain extracellular glucose were studied in the freely moving rat. These two variables were measured in the striatum during and following both mild tail pinch and restraint stress. Blood flow was monitored using a refinement of the hydrogen clearance technique that allowed repeated measurements at 5-min intervals. A slow stream of hydrogen was directed at the rat's snout for 10–20 s through lightweight tubing attached to the animal's head and detected at a chronically implanted platinum electrode. Extracellular glucose was monitored with microdialysis in a separate group of animals using an on-line, enzyme-based assay that provided 2.5-min time resolution. Mean striatal blood flow 24 h following implantation was $89.9 \pm 2.5 \text{ ml} \cdot (100 \text{ g})^{-1} \cdot \text{min}^{-1}$. A 5-min tail pinch caused flow to increase immediately to $169.5 \pm 20 \text{ ml} \cdot (100 \text{ g})^{-1} \cdot \text{min}^{-1}$. In contrast, there was no change in blood flow during restraint stress, although there was a small increase following the end of the stress. Significant increases in blood flow were also observed in the striatum during periods of eating and grooming. Extracellular glucose levels increased following both forms of stress, to a maximum of $170 \pm 22\%$ of baseline with restraint compared to $110 \pm 2\%$ with tail pinch. In both cases, the increase occurred after the stress had ended and persisted while blood flow returned to basal levels.

INTRODUCTION

Positron emission tomography (PET) studies in humans have suggested that a tight coupling exists between local energy metabolism and regional cerebral blood flow (rCBF)^{21,22}. Autoradiographic studies in animals also suggest a correlation between regional glucose utilisation and rCBF^{23,24}.

Brain extracellular glucose represents a balance between supply from the blood and cellular utilisation and these two processes were thought to be closely coupled in the brain^{8,13}. Thus, a change in neuronal activity would not be expected to cause a change in extracellular glucose levels. However, we have found that extracellular glucose levels monitored with microdialysis vary with local drug-induced changes in neuronal activity¹¹.

Microdialysis coupled with an on-line enzyme-based assay allowed extracellular glucose in the striatum to be monitored at 2.5-min intervals in the freely moving

rat⁴. Glucose supply is affected by a number of variables, including plasma concentration, rate of transport across the blood–brain barrier and blood flow²⁰. In view of the tight coupling between regional glucose utilisation and blood flow, we decided to measure changes in extracellular glucose during physiological activity and to monitor rCBF under the same conditions.

Due to methodological constraints there have been relatively few studies of rCBF in awake animals under physiological conditions^{9,16}. None of the available methods for measuring rCBF in small animals allows repeated measurements in the awake animal at high time resolution. Radioactive tracer techniques coupled with autoradiography provide good spatial resolution, but time course information requires the use of a large number of animals^{19,25}. Furthermore, frequent blood sampling means that unanaesthetised animals are often partially restrained¹⁵.

The measurement of hydrogen clearance is a well-

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established technique for determining rCBF^{25,26}. Hydrogen is administered by inhalation and monitored by amperometric detection at an electrode implanted in the brain. The rate of hydrogen clearance, once administration has stopped, is proportional to local blood flow²⁶. This method allows repeated measurements in the same animal and is also suitable for chronic recording over several days^{9,14}. However, this technique has been most extensively used in anaesthetised preparations because of the difficulties of controlling the administration of the hydrogen tracer. Previous attempts to adapt this technique for use in awake animals have involved confining the animals in a vented chamber through which a hydrogen-air mixture is passed at high volume flow rate^{6,9}. This gives poor time resolution and is potentially stressful.

Here we report a novel hydrogen delivery system that directs a slow stream of pure hydrogen at the rat's snout for 10–20 s, allowing rCBF measurements to be made at 5-min intervals in the undisturbed, freely moving animal. This adaptation of the hydrogen clearance technique has allowed us to monitor changes in striatal blood flow during normal behaviour and to evaluate the time course of changes in blood flow with two forms of mild stress. The changes in blood flow are compared with the variations in brain extracellular glucose observed under the same conditions. Some of these results have been reported in preliminary form¹⁰.

MATERIALS AND METHODS

Electrodes

The hydrogen detection electrode was of Teflon-coated 90% platinum/10% iridium wire (bare diameter, 50 μm ; coated diameter, 75 μm ; Clark Electromedical Equipment) with a 4-mm active length. To facilitate implantation, the wire was glued into a 20-mm length of plastic-coated silica tubing (o.d. 0.17 mm; Scientific Glass Engineering, UK) and a 14 mm section of stainless steel cannula (o.d. 0.5 mm; Goodfellow, UK). Only the platinum wire and 2 mm of the silica tubing penetrated the brain. The detection electrode was held at 250 mV vs. a chloridised silver wire (o.d. 0.325 mm) implanted in the cortex. Auxiliary and earth electrodes were also of 0.325 mm Teflon-coated silver wire (Clark Electromedical Equipment, UK). All electrodes were soldered to gold connectors, which were cemented into a six-pin plastic socket (both from Plastics One, USA).

Microdialysis probes

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

Surgery

Male Sprague–Dawley rats (200–300 g) were anaesthetised with a combination of fentanyl/fluanisone (0.25/0.8 mg/kg, i.p.) and midazolam (0.4 mg/kg i.p.) and placed in a stereotaxic frame. Body

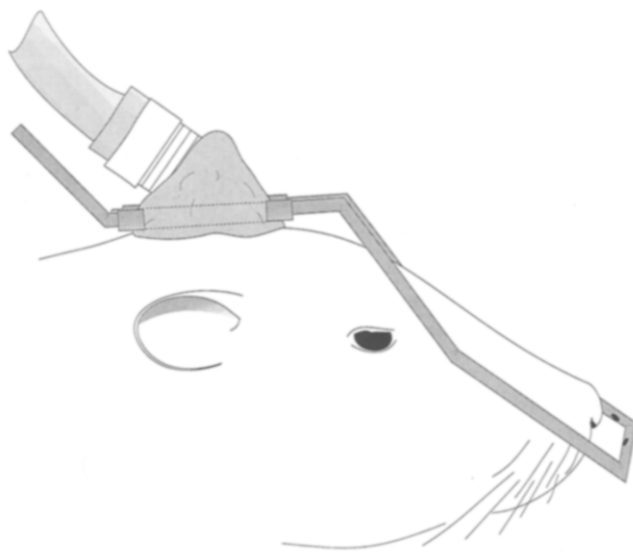


Fig. 1. Apparatus for delivering hydrogen to a freely moving rat. PEEK tubing is held in two channels in the dental acrylicate which secures the implanted electrodes and bent in order to position two 0.4-mm holes beneath the rat's nostrils. Details are given in the text.

temperature was maintained at 37°C with a heating pad (Sandown Scientific, UK). The detection electrode was implanted into the right striatum (coordinates, from bregma: A/P +1.0, M/L +2.5 and D/V from dura: –8.5 mm). The reference electrode was placed in the cortex and the auxiliary and earth electrodes were slipped between the skull and the dura at separate sites. The electrodes were fixed to the skull with screws and dental acrylicate. Two lengths of plastic tubing were imbedded in the dental acrylicate to provide channels for the hydrogen delivery system (see below).

Microdialysis probes were implanted in the right striatum in a separate group of animals. The active part of the dialysis membrane was placed in the same region of the striatum as the hydrogen detection electrode.

The animals were then allowed to recover from anaesthesia. Post-operative analgesia was provided in the form of a single injection of buprenorphine (0.1 mg/kg, s.c.) given immediately following the surgery. The rats were housed in large plastic bowls (Johnson's Garden Centre, Oxford), with free access to food and water. Experiments were carried out with the animal in its home bowl. The health of the animals was assessed following recovery according to published guidelines¹⁸ and all procedures were specifically licensed under the Animals (Scientific Procedures) Act, 1986.

Hydrogen delivery

Lightweight polyethyletherketone (PEEK) HPLC tubing (i.d. 1.6 mm, o.d. 3.2 mm; Anachem, UK) was passed through channels in the dental acrylicate and bent in such a way as to position two 0.4-mm holes about 5 mm beneath the rat's nostrils (see Fig. 1). This lightweight, rigid structure was well tolerated by the rats, which exhibited normal behaviour while it was in place. The PEEK tubing was connected through a flashback arrestor and a pressure regulator to a hydrogen cylinder (zero grade, British Oxygen, UK), with the gas flow controlled external to the rat's bowl. This arrangement allowed measurements to be made without disturbing the animal. The gas was administered for approximately 10–20 s, typically at 5-min intervals and at a flow rate of 150 ml/min.

Hydrogen detection and data acquisition

The platinum detection electrode was held at 250 mV vs. Ag^+/AgCl with a low-noise potentiostat of our own design (Biostat

II, EMS, Oxford). At this potential, detection of hydrogen is mass-transport limited and is not compromised by detection of other oxidisable species present in the brain²⁶. The current was digitised at 16-bit resolution through an NB-MIO-16X multifunction board (National Instruments Corp., USA) in a Macintosh IIfx computer and was controlled using LabVIEW software (National Instruments, USA). Current values were recorded by the computer at 0.5-s intervals. To further reduce noise, each value was an average of 75 determinations made at 2-ms intervals.

For each blood flow measurement, the LabVIEW program was started and the baseline was recorded for 5 s. Hydrogen was then administered until the current had increased by 30–80 nA. The decay curve was collected for a 3-min period. The first 30–60 s of the curve were neglected and the remaining 120–240 s were transferred to an analysis program. Here a first-order analysis plot of $\ln(i(t) - i_{\text{baseline}})$ vs. time t was performed. If a linear plot was obtained, the slope was converted to blood flow according to the following formula (assuming a partition coefficient of unity):

$$rCBF \text{ (ml} \cdot \text{(100 g)}^{-1} \cdot \text{min}^{-1}) = \text{slope (s}^{-1}) \cdot 60 \cdot 100 \text{ g} \quad (1)$$

When the semilog plot showed a clear break in the slope, the decay curves before and after the break point were analysed separately.

Analysis of glucose

The dialysate was analysed for glucose using a flow injection enzyme-based assay that is described in detail elsewhere⁴. Briefly, glucose oxidase and horseradish peroxidase (Boehringer-Mannheim, FRG) were immobilised on 10 μm silica beads, which were then packed into a small bed. A phosphate buffer containing the electrochemical mediator species ferrocene (composition (in mM): Na_2HPO_4 100, EDTA 1, ferrocene monocarboxylic acid 2, adjusted to pH 7.0, with 0.05% Kathon CG added to inhibit bacterial growth) was pumped through the bed at 0.5 ml/min by an HPLC pump (Pharmacia LKB 2150). Glucose was oxidised by glucose oxidase, producing hydrogen peroxide. Horseradish peroxidase converted this to water and was regenerated by the oxidation of the ferrocene species present in the buffer. The ferricinium produced was detected

by reduction at a downstream electrode held at 0.0 V vs. Ag/AgCl by a potentiostat of our own design.

Experimental conditions

At least 90 min prior to the start of each blood flow experiment, the rat was placed in a fume cupboard, in its home bowl. The hydrogen delivery tubing was put in position and connected to the gas supply. The electrodes were connected to the potentiostat through a flexible cable and swivel (Plastics One, USA) which allowed the animal free movement. Once the background current had stabilised and the rat had become accustomed to the new conditions, blood flow measurements were made at 5-min intervals.

The microdialysis experiments were carried out following a 24 h recovery period. The microdialysis probes were connected to a microinfusion pump (CMA Microdialysis) through a liquid swivel, allowing the animals free movement. The probes were perfused with an artificial cerebrospinal fluid (aCSF) (composition (in mM): NaCl 147, KCl 4.0, CaCl_2 1.2, MgCl_2 1.0), at 2 $\mu\text{l}/\text{min}$.

The outflow of the microdialysis probe was connected through low dead volume FEP tubing (CMA Microdialysis) to a 22-gauge square ended injection syringe needle (Hamilton, Switzerland). The needle was inserted into the VISF-1 injection port of an HPLC valve (Valco C6W) mounted as part of a CMA/160 on-line injector (CMA Microdialysis). This arrangement allowed 5 μL dialysate samples to be automatically injected onto the enzyme packed bed, providing on-line analysis of glucose at 2.5-min intervals.

Physiological stimulation

Two forms of mild stress were studied. In the first, gentle pressure was applied to the rat's tail for 5 min by means of a paper clip attached about 3 cm from the tip. This tail pinch produces a stereotyped gnawing behaviour². The rat vigorously chewed a wooden stick held by the observer until the clip was removed.

The effect of restraint stress was also examined. The rat was restrained for 10 min in a transparent, ventilated box. The box prevented any movement, but did not compromise respiration and involved only minimal handling of the animal at the start of the procedure.

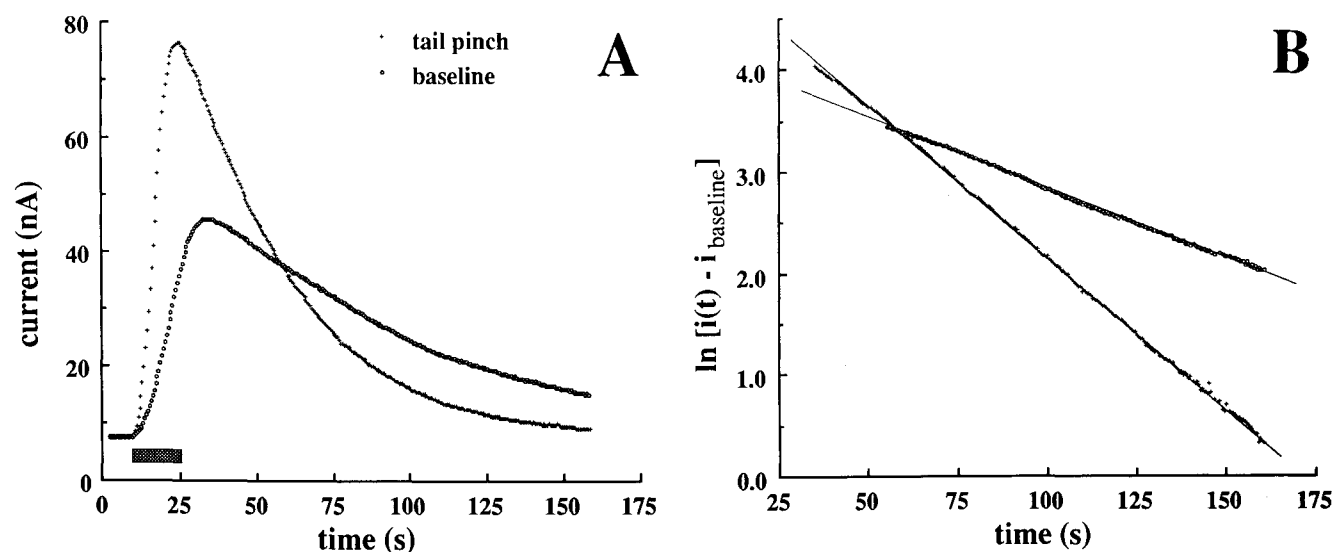


Fig. 2. A: two examples of hydrogen clearance curves from the same rat, under conditions of basal and tail pinch-stimulated blood flow. The dark bar indicates the period of hydrogen administration. Current is plotted at 0.5-s intervals. B: the analysis of the curves shown in Fig. 2A. The expression $\ln(i(t) - i_{\text{baseline}})$ was plotted against time t , and the data fitted by linear regression. The mean squared error for the basal blood flow data was 0.00011 and that for the tail pinch-stimulated blood flow data was 0.0004. The slope of each line was converted to CBF as described in the text. The basal striatal blood flow was $83.9 \text{ ml} \cdot \text{(100 g)}^{-1} \cdot \text{min}^{-1}$ and the tail pinch-stimulated blood flow was $178.9 \text{ ml} \cdot \text{(100 g)}^{-1} \cdot \text{min}^{-1}$.

TABLE I

None of the values are significantly different from each other (unpaired Student's *t*-test, $P > 0.05$)

Time post-implantation (h)	mean basal CBF \pm S.E.M. ($\text{ml} \cdot (100 \text{ g})^{-1} \cdot \text{min}^{-1}$)	n
6 (transient administration)	93.3 \pm 5.9	5
6 (saturation)	86.1 \pm 9.8	5
24	89.9 \pm 2.5	5
48	85.8 \pm 3.1	5

RESULTS

Measurement of rCBF

The background current was very stable and did not drift during the 3–4 h experiments. It was typically 6–7 nA, although somewhat higher 6 h post-implantation. The transient administration of hydrogen led to a 30–80 nA increase in current. The use of a three-electrode system and low-noise potentiostat gave very quiet signals (see Fig. 2A).

Fig. 2A shows typical hydrogen clearance curves measured under basal and tail pinch-stimulated conditions in the same animal. Clearance curves were generally well fitted by a single exponential, as shown in Fig. 2B. Blood flow values did not depend on the absolute size of the current, which varied between 30 and 80 nA depending on the efficiency of the hydrogen delivery.

Hydrogen clearance measurements are usually made following the administration of hydrogen to saturation levels. We therefore compared the blood flow estimates based on transient hydrogen inhalation with those determined following saturation at 6 h post-implantation (Table I). Steady state conditions were reached within 4–5 min of gas administration and produced currents 15–20-fold higher than those observed following 10–20 s of hydrogen administration. The mean blood flow determined with saturation levels of hydrogen was not significantly different from that measured following transient hydrogen inhalation ($P > 0.05$, unpaired Student's *t*-test; $n = 5$).

Basal rCBF and extracellular glucose in the undisturbed rat

Mean values for basal striatal blood flow at 6, 24 and 48 h following electrode implantation are presented in Table I. The values were not significantly different at any of these time points ($P > 0.05$, unpaired Student's *t*-test; three or four measurements in five rats).

Microdialysis experiments were carried out at 24 h post-implantation. The basal dialysate glucose concentration was $182 \pm 12 \mu\text{M}$ ($n = 10$).

Effect of tail pinch on extracellular glucose and rCBF

Application of a 5-min tail pinch caused an increase in dialysate glucose levels of $110 \pm 2\%$ of baseline ($n = 6$), which occurred immediately after the stimulus was applied. Levels returned toward baseline only to show a second prolonged elevation of similar magnitude that was still significant at 32.5-min post tail pinch (Fig. 3A).

Tail pinch caused an immediate increase in striatal blood flow which reached a maximum of $169.5 \pm 20 \text{ ml} \cdot (100 \text{ g})^{-1} \cdot \text{min}^{-1}$ (eight tail pinches in five rats) during the stimulation. This represents an increase of $198 \pm 16\%$ of baseline. Blood flow returned to baseline within 15 min of the tail pinch and remained at basal levels for at least 55 min.

Effect of restraint stress on extracellular glucose and rCBF

Ten minutes of restraint stress resulted in significant and prolonged increases in the glucose levels in striatal dialysate, reaching $170 \pm 22\%$ of baseline ($n = 4$). Glucose levels began to rise slowly during the stress, but

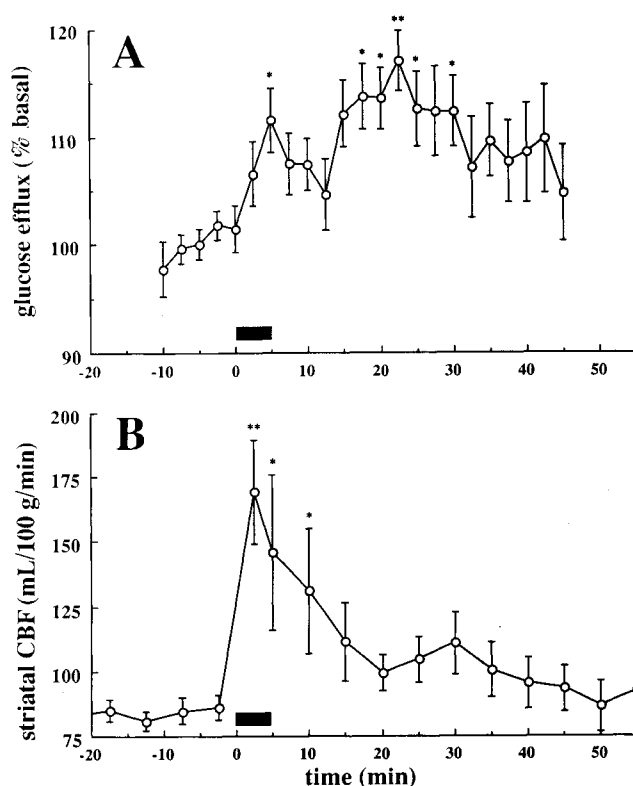


Fig. 3. A: effect of a 5-min tail pinch (dark bar) on dialysate glucose ($n = 6$) levels in the striatum. Values are mean \pm S.E.M., expressed as a percentage of the mean baseline. * $P < 0.05$, ** $P < 0.01$ Mann-Whitney *U*-test vs. last baseline point. B: the effect of a 5-min tail pinch (open bar) on striatal blood flow. Values are mean \pm S.E.M. for eight tail pinches in five rats, 24–48 h following implantation. ** $P < 0.01$, * $P < 0.05$, Mann-Whitney *U*-test vs. the last baseline value.

were not significantly above baseline until 15 min. This increase in dialysate glucose persisted for 20 min and then gradually returned to baseline (Fig. 4A).

There was a marked difference in the effect on striatal blood flow of restraint stress and tail pinch. During the restraint, there was no significant change in blood flow compared to baseline. In some rats, a small increase in blood flow occurred once they were released (Fig. 4B). The maximum significant increase was $123 \pm 13\%$ of basal values. A brief period of grooming frequently followed restraint stress, which may have partly contributed to this increase. The mean striatal blood flow was significantly above baseline values at 20 and 30 min post-stimulus.

rCBF during normal behaviour

The rats exhibited normal behaviour during the experiments, allowing the blood flow measurements to be made during periods of spontaneous grooming and eating (Fig. 5). Striatal blood flow increased by $13.3 \pm 2.6 \text{ ml} \cdot (100 \text{ g})^{-1} \cdot \text{min}^{-1}$ above baseline during eating ($n = 3$), while grooming provoked a somewhat larger increase of $19.5 \pm 3.4 \text{ ml} \cdot (100 \text{ g})^{-1} \cdot \text{min}^{-1}$ (nine measurements in five rats). This represents an increase of

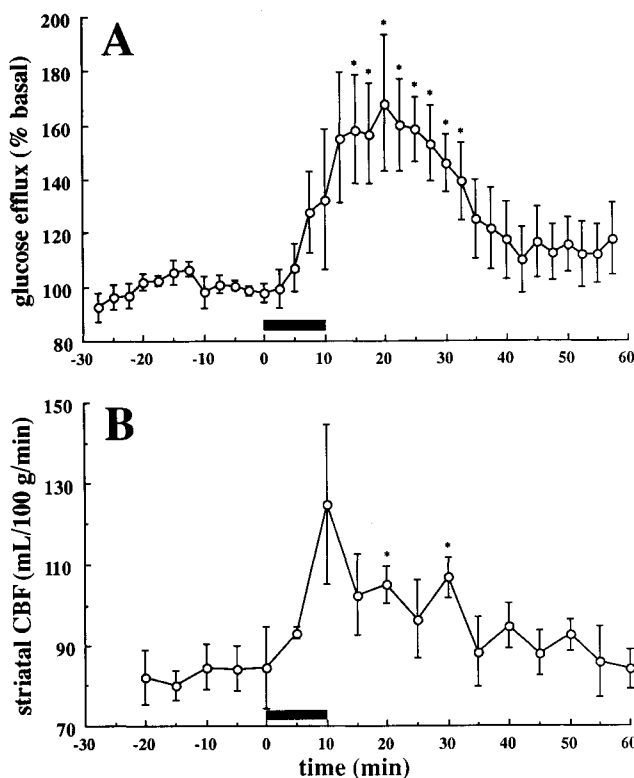


Fig. 4. A: effect of 10 min of restraint (dark bar) on dialysate glucose ($n = 4$) levels in the striatum. Values are mean \pm S.E.M., expressed as a percentage of the mean baseline. * $P < 0.05$, Mann-Whitney U -test vs. last baseline point. B: the effect of a 10-min period of restraint stress (dark bar) on striatal blood flow. Values are mean \pm S.E.M. for 6 in four rats, 24–48 h following implantation. * $P < 0.05$, paired Student's t -test vs. the last baseline value.

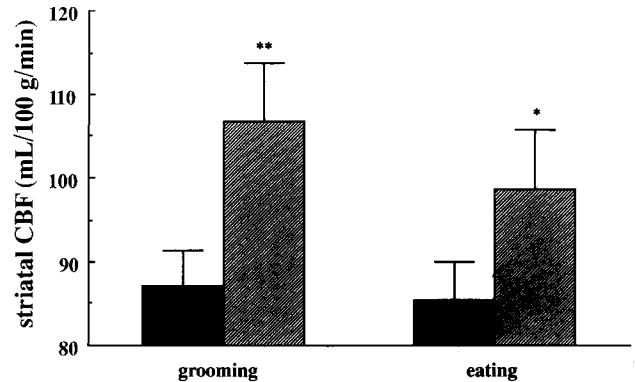


Fig. 5. The mean striatal blood flow during periods of spontaneous eating ($n = 3$) and grooming ($n = 5$). Values are mean \pm S.E.M. Stimulated blood flow values were compared to the preceding baseline value in each case, ** $P < 0.01$, * $P < 0.05$ (paired Student's t -test).

$115 \pm 2\%$ and $122 \pm 3\%$ above basal values, respectively.

DISCUSSION

Basal blood flow values measured in the freely moving rat with this adaptation of the hydrogen clearance technique agreed closely with literature values. At 6 h post-implantation, mean striatal blood flow was $93.3 \pm 5.9 \text{ ml} \cdot (100 \text{ g})^{-1} \cdot \text{min}^{-1}$. This compares well with the value of $104.5 \pm 6.9 \text{ ml} \cdot (100 \text{ g})^{-1} \cdot \text{min}^{-1}$ determined 6 h post-surgery with the [^{14}C]iodoantipyrine technique in awake rats¹⁹.

The spreading depression that accompanies the implantation of the detecting electrode has been shown to disturb CBF for 2–3 h after surgery, making the hydrogen clearance technique inappropriate for acute blood flow measurements. However, between 6 and 24 h the hydrogen clearance technique gives values for rCBF in good agreement with those determined by radioactive tracer methods²⁵. Basal striatal blood flow was not different at 6, 24 and 48 h, in agreement with the finding of Jackowski et al. (1989) that consistent results could be obtained with chronically implanted platinum electrodes for up to 7 days¹⁴.

The hydrogen clearance technique is most often used in anaesthetised preparations and hydrogen is normally administered to saturation levels, which takes several minutes²⁶. One study using brief (1–3 min) inhalation reported a very fast initial component to the clearance curves, which was attributed to equilibration of the hydrogen between different tissue compartments¹².

The transient administration of hydrogen used in this study resulted in peak currents that were about one-fifteenth those obtained with saturation. These clearance curves were generally well described by a

single exponential (Fig. 2A). We compared the blood flow values determined with transient hydrogen administration with those measured following the administration of hydrogen to saturation levels. Interestingly, the decay curve following saturation often had a much faster initial component during the first 60 s of clearance. The blood flow estimated from the slow component was not significantly different from the flow determined following transient hydrogen administration (Table I). This finding confirmed that brief administration of hydrogen was suitable for measuring CBF.

Reducing the hydrogen administration time to 10–20 s made it possible to make blood flow measurements with 5-min time resolution. This measurement interval is considerably smaller than that reported for either the more conventional hydrogen clearance method in awake rats (15–60 min)^{6,9} or PET (10 min)¹⁷. Furthermore, it is on a similar scale to the on-line assay for dialysate glucose used in this study, allowing transient changes in extracellular glucose to be compared with blood flow.

The rats quickly habituated to the presence of the hydrogen delivery tube and slept, ate and groomed normally throughout the experiments, including during the periods of hydrogen administration. The gas delivery was controlled external to the rat's bowl, allowing measurements to be made without disturbing the animal. Thus, it was possible to measure blood flow during spontaneous behaviour, as well as to determine undisturbed basal values for comparison with those obtained under conditions of mild stress. As the experiments took place during the sleep cycle of the animals, most of the measurements of basal blood flow were made while the rat rested quietly. Under these conditions, striatal blood flow was very constant (Figs. 3B, 4B). However, blood flow was very sensitive to changes in behaviour, increasing by 115–122% during periods of eating or grooming (Fig. 5).

There were no striking changes in basal glucose levels during normal behaviour in the freely moving rat. However, both tail pinch and restraint stress provoked long-lasting increases in dialysate glucose, with restraint stress having a much larger effect.

The application of a mild tail pinch for 5 min resulted in an immediate increase in striatal blood flow (Fig. 4). Regional CBF has been shown to be closely linked to local glucose metabolism and hence functional activity²⁴. The immediate, large increase in blood flow observed during tail pinch suggests that this stimulus leads to intense activity in the striatum. This is in agreement with the increase in a number of neurochemicals, including dopamine, ascorbate⁵ and glutamate¹ measured in the striatum during this stimulus.

Extracellular glucose briefly increased following tail pinch, with a second, prolonged increase occurring between 15 and 32.5 min. The first increase closely follows the sharp rise in blood flow and falls toward baseline as blood flow falls. However, the second, prolonged increase in glucose levels occurs in the face of decreasing blood flow. It appears that glucose supply is not challenged by enhanced utilisation during physiological stimulation, although local drug-induced increases in neuronal energy demands lead to a decrease in extracellular glucose¹¹.

Restraint stress led to a much larger increase in extracellular glucose and did not provoke the transient rise and fall seen immediately following tail pinch; instead, striatal glucose levels increased slowly during the stress and were significantly above baseline between 15 and 32.5 min.

During the 10 min of restraint stress, striatal CBF did not differ from control levels. However, in many animals blood flow increased once they were released from the restraining box. This increase may have been partly a result of the grooming that frequently followed restraint stress. The maximum increase was $123 \pm 13\%$, which is similar to the increase of $122 \pm 3\%$ seen during spontaneous grooming.

The small increase in blood flow which occurred following restraint closely follows the changes in extracellular glucose. The finding that blood flow changed very little with restraint implies that there was little change in local neuronal activity in the striatum during this stress. This is in agreement with 2-deoxyglucose autoradiography studies which found no change in striatal glucose utilisation during restraint⁷.

There have been several, conflicting reports on the effect of restraint stress on rCBF in rats^{7,15,19}. Most have reported little, if any, change in rCBF during restraint. However, these studies have been performed with the [¹⁴C]iodoantipyrine technique, which may impose some degree of stress in itself¹⁵. In the experiment reported here, 10 min of restraint stress followed several baseline measurements during which the rats were completely undisturbed. Our results confirm that there is no change in striatal blood flow during restraint stress, but indicate that there is a slight increase following the stress. Previous studies did not measure blood flow after the restraint period.

Thus, restraint stress provoked small changes in blood flow and large increases in glucose, while tail pinch was accompanied by a substantial increase in blood flow and only slight changes in glucose levels. In both cases, elevated blood flow was associated with the initial increase in glucose levels. However, the prolonged increase in dialysate glucose which followed

both stresses was not linked to higher blood flow. Mild stress induces an increase in plasma glucose of 1–3 mM which can persist for up to 2 h³. It is likely that this hyperglycemia contributes to the over-supply of brain extracellular glucose which follows tail pinch and restraint stress.

Acknowledgements. We thank the SERC (UK) and the Wellcome Trust for financial support. LKF is in receipt of a Rhodes Scholarship. We thank Dr. Marianne Fillenz for provision of laboratory space and many helpful discussions. We also thank Dr. David Paterson and Dr. Derek Bergel for helpful discussions on the measurement of blood flow. The restraint box was designed and built by Mr. Abdolvahab Vahabzadeh.

REFERENCES

- Albery, W.J., Boutelle, M.G. and Galley, P.T., The dialysis electrode – a new method for in vivo monitoring, *Chem. Comm.*, 1992 (1992) 900–901
- Antelman, S.M., Szechtman, H., Chin, P. and Fisher, A.E., Tail pinch-induced eating, gnawing and licking behavior in rats: dependence on the nigrostriatal dopamine system, *Brain Res.*, 99 (1975) 319–337.
- Bialik, R.J., Smythe, J.W., Sardelis, M. and Roberts, D.C.S., Adrenal demedullation blocks and brain norepinephrine depletion potentiates the hyperglycemic response to a variety of stressors, *Brain Res.*, 502 (1989) 88–98.
- Boutelle, M.G., Fellows, L.K. and Cook, C., Enzyme packed bed system for on-line measurement of glucose, glutamate and lactate in brain microdialysate, *Anal. Chem.*, 64 (1992) 1790–1794.
- Boutelle, M.G., Zetterström, T., Pei, Q., Svensson, L. and Fillenz, M., In vivo neurochemical effects of tail pinch, *J. Neurosci. Methods*, 34 (1990) 151–157.
- Bray, L., Lartaud, I., Muller, F., Atkinson, J. and Capdeville, C., Effects of the angiotensin I converting enzyme inhibitor perindopril on cerebral blood flow in awake hypertensive rats, *Am. J. Hypertens.*, 4 (1991) 246S–252S.
- Bryan, R.M., Cerebral blood flow and energy metabolism during stress, *Am. J. Physiol.*, 259 (1990) H269–H280.
- Cremer, J.E., Cunningham, V. and Seville, M.P., Relationships between extraction and metabolism of glucose, blood flow and tissue blood volume in regions of rat brain, *J. Cereb. Blood Flow Metab.*, 3 (1983) 291–302.
- Endo, Y., Jinnai, K., Endo, M., Fujita, K. and Kimura, F., Diurnal variation of cerebral blood flow in rat hippocampus, *Stroke*, 21 (1990) 1464–1469.
- Fellows, L.K., Boutelle, M.G. and Fillenz, M., Brain energy metabolism studied by monitoring extracellular glucose and lactate with microdialysis in the awake rat, *J. Physiol.*, in press.
- Fellows, L.K., Boutelle, M.G. and Fillenz, M., Extracellular brain glucose levels reflect local neuronal activity: a microdialysis study in awake, freely moving rats, *J. Neurochem.*, 69 (1992) 2141–2147.
- Halsey, J.H., Capra, N.F. and McFarland, R.S., Use of hydrogen for measurement of regional cerebral blood flow – problem of intercompartmental diffusion, *Stroke*, 8 (1977) 351–357.
- Hawkins, R.A., Transport of essential nutrients across the blood–brain barrier of individual structures, *Fed. Proc.*, 45 (1986) 2055–2059.
- Jackowski, A., Crockard, A., Ross-Russell, R. and Burnstock, G., Serial determinations of regional cerebral blood flow in the rat using simple chronically implanted platinum wire microelectrodes, *J. Neurosci. Methods*, 30 (1989) 133–139.
- Lasbennes, F., Lestage, P., Bobillier, P. and Seylaz, J., Stress and local cerebral blood flow: studies on restrained and unrestrained rats, *Exp. Brain Res.*, 63 (1986) 163–168.
- Ledoux, J.E., Thompson, M.E., Iadecola, C., Tucker, L.W. and Reis, D.J., Local cerebral blood flow increases during auditory and emotional processing in the conscious rat, *Science*, 221 (1983) 576–578.
- Mazziota, J.C., Grafton, S.T. and Woods, R.C., The human motor system studied with PET measurements of cerebral blood flow: topography and motor learning. In N.A. Lassen, O.H. Ingvar, M.E. Raichle and L. Friberg (Eds.), *Brain Work and Mental Activity: Quantitative Studies with Radioactive Tracers*, Munksgaard, Copenhagen, 1991, pp. 280–290.
- Morton, D.B. and Griffiths, P.H.M., Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment, *Vet. Rec.*, 116 (1985) 431–436.
- Ohata, M., Fredericks, W.R., Sundaram, U. and Rapoport, S.I., Effects of immobilization stress on regional cerebral blood flow in the conscious rat, *J. Cereb. Blood Flow Metab.*, 1 (1981) 187–194.
- Robinson, P.J. and Rapoport, S.I., Glucose transport and metabolism in the brain, *Am. J. Physiol.*, 250 (1986) R127–R136.
- Roland, P.E., Eriksson, L., Stone-Elander, S. and Widen, L., Does mental activity change the oxidative metabolism of the brain?, *J. Neurosci.*, 7 (1987) 2372–2389.
- Siesjö, B.K., Cerebral circulation and metabolism, *J. Neurosurg.*, 60 (1984) 883–908.
- Sokoloff, L., Relationship among local functional activity, energy metabolism and blood flow in the central nervous system, *FASEB J.*, 40 (1981) 2311–2316.
- Sokoloff, L., Relationship between functional activity and energy metabolism in the nervous system: whether, where and why? In N.A. Lassen, O.H. Ingvar, M.E. Raichle and L. Friberg (Eds.), *Brain Work and Mental Activity: Quantitative Studies with Radioactive tracers*, Munksgaard, Copenhagen, 1991, pp. 52–64.
- Tomida, S., Wagner, H.G., Klatzo, I. and Nowak, T.S., Jr, Effect of acute electrode placement on regional CBF in the gerbil: a comparison of blood flow measured by hydrogen clearance, [³H]nicotine and [¹⁴C]iodoantipyrine techniques, *J. Cereb. Blood Flow Metab.*, 9 (1989) 79–86.
- Young, W., H₂ clearance measurement of blood flow: a review of technique and polarographic principles, *Stroke*, 11 (1980) 552–564.