Acta Physiol Scand 1999, **167**, 275–284

The role of astrocytes and noradrenaline in neuronal glucose metabolism

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ABSTRACT

In the classical model the energy requirements during neuronal activation are provided by the delivery of additional glucose directly into the extracellular compartment that results from the increase in local cerebral blood flow (rCBF). The present review proposes that astrocytes play a key role in the response to neuronal activation. Arginine for the synthesis of NO, which has a major role in the increase in rCBF, is released from astrocytes in response to stimulation of astrocytic glutamate receptors. The increased delivery of glucose by the blood stream enters astrocytes, where some of it is converted to glycogen. During neuronal activation there is a decrease in extracellular glucose owing to increased utilization followed by a delayed increase; this results from stimulation of astrocytic β-adrenergic receptors, which leads to a breakdown of glycogen and the export of glucose.

Keywords astrocytes, glucose, neurones, noradrenaline.

Received 8 February 1999, accepted 13 May 1999

The brain, which makes up 2% of the body weight, receives 15% of the cardiac output (Sokoloff 1960). The role of the cerebral blood supply is the delivery to the brain of metabolic substrates for the production of energy and the removal of waste products. As there are no energy reserves, a constant supply of oxygen and glucose, the main metabolic substrates, is required and any interruption of the blood supply rapidly produces profound and irreversible changes of brain function (Scheinberg & Joyne 1952, Choi 1990). An increase in neuronal activity is accompanied by an increase in energy requirement. There has been much recent research on the mechanisms for the provision of this additional energy.

In this review the hypothesis proposed is that glucose from the blood is not delivered directly to the extracellular environment of the neurones but enters the astrocytes from which its release is regulated by the activation of noradrenergic β-receptors.

The brain is contained in a bony cranium and cerebral tissue, cerebrospinal fluid and blood are incompressible; the volume of the cerebral circulation is therefore kept constant. However, global blood flow can be increased by an increase in the velocity of flow and local cerebral blood flow can be increased by a process of redistribution, leading to a local increase in both volume and velocity of flow in selected areas of the brain.

The idea that cerebral blood flow varies with brain activity was suggested towards the end of the last century (Raichle 1998) but Roy & Sherrington (1890) were the first to carry out experimental investigations.

Localized blood flow is controlled by the state of constriction of the small arterioles, which regulates the distribution of blood to a capillary bed. Arteriolar constriction is brought about by contraction of myocytes which in turn depends on intracellular calcium $[Ca^{2+}]_i$. Membrane potential of smooth muscle cells is a major determinant of cytosolic $[Ca^{2+}]_i$ and thus vascular tone. Potassium channels play an important role in the control of smooth muscle membrane potential. However, some vasoactive agents can produce contraction or relaxation of cerebral blood vessels without a change in membrane potential.

Much of the study of the control of cerebral blood vessels has been carried out *in vitro*, either using the patch clamp technique on isolated myocytes, to

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investigate the role of ion channels on membrane potential, or the measurement of membrane potential in intact but isolated vascular rings or segments. Such studies have demonstrated the presence of four types of K^+ channels. The characteristics of these K^+ channels and their role in the regulation of cerebral vascular tone have been reviewed by Faraci & Sobey (1998). The vasoactive stimuli which regulate these K^+ channels include nitric oxide (NO), cAMP, K^+ ions; to these has recently been added epoxyeicosatrienic acid (EET), a product of Cytochrome P450 metabolism of arachidonic acid. NO can produce relaxation by either soluble cGMP-dependent or soluble cGMP-independent mechanisms. All these vasoactive mechanisms can be activated by a wide variety of transmitters, metabolites and drugs.

In order to determine the functional importance of these mechanisms, experiments have been carried out *in vivo*: where the changes in cerebral blood flow, that result from the application of drugs which activate or inhibit these various mechanisms are monitored. The presence of multiple cell types makes it difficult to distinguish direct from indirect effects in these experiments.

Both hypoxia and hypercapnia lead to an increase in cerebral blood flow and there have been numerous studies of the factors which control cerebral blood flow during these two conditions. Hypoxia and hypercapnia are relatively easy to manipulate and produce changes in blood flow. The increase of local cerebral blood flow during neuronal activation, called functional hyperaemia, is much more difficult to investigate. Whereas hypoxia and hypercapnia cause changes in global cerebral blood flow, whose duration is determined by the time course of the changes in respiratory gases, functional hyperaemia is triggered by a localized increase in neuronal activity, which is usually short-lived and produces very localized changes in cerebral blood flow. These require techniques for measurement of regional cerebral blood flow.

REGIONAL CEREBRAL BLOOD FLOW (rCBF)

A widely used model of changes in rCBF in response to neuronal activation by a physiological stimulus is the rodent whisker barrel cortex. This has the advantage that the whisker barrel cortex accounts for 20% of the somatosensory area I in rodents and each sinus hair is represented by a column of cortical cells supplied by one penetrating artery (Welker 1971). The changes in rCBF following whisker stimulation have been investigated with a wide variety of techniques, which include the use of radioactive and volatile tracers, Doppler flowmetry and videomicroscopy (Woolsey *et al.* 1996). Although these methods vary in their spatial and temporal resolution, they all show an increase in rCBF in the activated region of the cortex and some methods show a decrease in the neighbouring non-activated cortical region. For an accurate determination of the time course of the change in rCBF laser–Doppler flowmetry (LDF) is one of the most useful techniques as it gives a continuous record and has a time resolution of 200 ms. Using this technique the rCBF increase produced by whisker stimulation shows an initial peak within 2–3 s after onset of stimulation, a sustained plateau throughout the 60-s period of stimulation and a return to near baseline level within 2–3 s after the end of stimulation (Lindauer *et al.* 1993). This rapid time course has to be taken into consideration in any proposed mechanism for the increase in rCBF during neuronal activation.

Astrocytes

Other important considerations are the anatomical relationships of the small cerebral vessels. Astrocytes are the most predominant type of glial cells in the CNS and constitute over 50% of the cell mass in the brain. They have an important role in a variety of brain functions and their morphological relationship to both neurones and cerebral blood vessels is therefore of considerable importance.

Astrocytes, coupled by gap junctions, form a network through which ions and small molecules can move freely (Gutnick *et al.* 1981). Evidence from primary cortical cultures has demonstrated rapid communication between neurones and astrocytes (Murphy *et al.* 1993, Nedergaard 1994), attributed to the existence of gap junctions between neurones and astrocytes by some (Nedergaard 1994) although denied by others (Murphy *et al.* 1993). Ultrastructural studies have shown that astrocytes contain glycogen granules, their endfeet ensheath parenchymal microvessels and they also send processes which surround synaptic complexes. (Peters *et al.* 1991). More recent electron microscopic studies have shown that the continuity of the atsrocytic endfeet surrounding the cerebral vessels is interrupted by noradrenergic nerve terminals (Papsapalas & Papadopoulos 1996). Immunostaining has revealed that endothelial cells carrying GLUT1 glucose transporters are enveloped by rosette-shaped endfeet derived from one or more astrocytes. (Kacem *et al.* 1998). The astrocytic gap junctions can be identified either by morphological criteria or with anti-Connexin43 antibodies. Immunocytochemical staining reveals a honeycomb meshwork of astrocytic endfeet surrounding small blood vessels. The gap junctions between the astrocytic endfeet allow the passage not only of ions but also metabolic

substrates such as glucose and lactate (Giaume *et al.* 1997) and may act as a diffusion barrier between the blood vascular system and the extracellular compartment.

Neurochemical changes

Neuronal activation by the application of physiological stimuli leads to a number of changes in the extracellular compartment which are potential mechanisms for the regulation of rCBF.

One of the earliest changes is a rise in $[K^+]_e$. The use of ion-selective electrodes has shown that repetitive electrical nerve stimulation at 10–100 Hz elicits increases in $[K^+]_e$ of 2.0–3.5 mM (Sykova *et al.* 1992).

As glutamate is the main excitatory transmitter, activation of a brain area will involve the release of glutamate. The use of microdialysis has shown that neuronal activation by application of physiological stimuli, such as induced grooming, is followed by a rise in the extracellular concentration of glutamate (Miele *et al.* 1996). Overflow from the synaptic cleft of glutamate from the vesicular store is one possible source for the rise in extracellular glutamate. However, the demonstration that such glutamate release is not abolished by local infusion of TTX or Ca^{2+} channel blockers suggests that there must be other mechanisms and sources of glutamate release, astrocytes being one possible source (Miele *et al.* 1996, Timmerman & Westerink 1997).

Increase in rCBF during neuronal activity

Rise in $[K^+]_e$

The rise in $[K^+]_e$ has long been regarded as the agent responsible for the increase in rCBF following neuronal activation. The efflux of K^+ during electrical activity of neurones would produce a very much greater increase in $[K^+]_e$ than the observed increase of 2.5–3 mM, if it were not for clearance mechanisms. Astrocytes have an important role in spatial buffering (Gardner-Medwin 1983). Their high K^+ permeability allows them to reduce local accumulation by uptake of K^+, which is then distributed through the electrically coupled network of astrocytes to other parts of the extracellular space, for eventual return to the neurones. This redistribution involves a number of ion channels as well as the Na^+/K^+ pump (Amedee *et al.* 1997).

The high density of potassium channels in astrocytic endfeet (Newman 1986) has raised the possibility that release of K^+ from these endfeet could regulate cerebral blood flow. The results of computer simulations of K^+ dynamics in the brain indicate that the release of K^+ from astrocytic endfeet raises perivascular K^+ concentration much more rapidly and to higher levels than does diffusion of K^+ through the extracellular space (Paulson & Newman 1987). Experiments with isolated cerebral arterioles show that K^+ dilates these vessels by a mechanism which, blocked by barium or caesium, is caused by the opening of K^+ channels, the most likely being the inwardly rectifying K^+ channels (Quayle *et al.* 1993). In slices of guinea-pig olfactory cortex, stimulation of the olfactory tract leads to an uptake of K^+ into astrocytes following the stimulation-induced rise in $[K^+]_e$; this uptake is blocked in the presence of Ba^{2+} (Ballanyi *et al.* 1987).

Attempts have been made to assess the importance of these mechanisms *in vivo*. Local surface electrical stimulation of the cortex causes an increase in rCBF, in $[K^+]_e$ and in astroglial $[K^+]$. During Ba^{2+} superfusion the stimulated increase in astroglial $[K^+]$ is diminished but the stimulated increase in rCBF and $[K^+]_e$ is slightly enhanced (Kraig & Iadecola 1989).

Stimulation of the fastigial nucleus of the cerebellum increases cortical CBF by about 200% with no change in local metabolism (Nakai *et al.* 1983). When changes in $[K^+]_e$ were monitored during such stimulation, increases were found to range from 0.5–2.9 mM and had a very much more restricted distribution than the increase in CBF. In order to see whether changes in $[K]_e$ could account for the increase in CBF, the cortex was superfused with a 20 mM K solution: this produced an increase in CBF of only 17 ± 2%. (Iadecola & Kraig 1991).

These findings argue against a role for $[K]_e$ in the stimulated increase in rCBF.

NO

An alternative mechanism for the increase in rCBF is the mediation by NO whose release is triggered by the action of glutamate. Of the three forms of nitric oxide synthetase (NOS), neuronal NOS (nNOS) is constitutively expressed in neurones and is activated by Ca^{2+}/calmodulin. Immunocytochemical studies have shown that nNOS in the cerebral cortex is found in a heterogeneous morphological group of neurones, which make up no more than 0.2–0.3% of the total neuronal population. One of the chemical characteristics of these nNOS containing neurones is the high degree of co-localization with GABA and the peptides somatostatin and NPY (Estrada & DeFelipe 1998).

In vivo NO is a powerful vasodilator (Marshall *et al.* 1988) and regulates resting cerebral blood flow (Faraci 1990, Rosenblum *et al.* 1990). Stimulation of glutamatergic NMDA receptors causes release of NO, dilatation of cerebral blood vessels (Faraci & Breese 1993) and an increase in cerebral blood flow (Yang & Chang 1998). nNOS, mainly found in neurones, requires arginine, which is localized in astrocytes. Arginine is released from brain slices and astrocytic cultures by glutamate acting on non-NMDA ionotropic glutamate receptors (Grima *et al.* 1997).

Administration of N^{ω}-substituted arginine analogues, which inhibit NOS stereospecifically and competitively, decrease resting cerebral arterial and arteriolar diameter (Faraci 1990, Rosenblum *et al.* 1990, Faraci & Breese 1993) and resting cerebral blood flow (Tanaka *et al.* 1991, Iadecola 1992, Dirnagl *et al.* 1993). Electrical stimulation of the rat sciatic nerve (Northington *et al.* 1992), or vibrissal stimulation (Amedee *et al.* 1997), produce an increase in cerebral blood flow in the somatosensory cortex, which is abolished by NOS inhibitors. NOS inhibitors have no effect on basal or stimulated cerebral metabolism (Cholet *et al.* 1997). The use of selective glutamatergic receptor antagonists has demonstrated the critical role of astrocytes, because the release of arginine from astrocytes controls neuronal NO synthesis and release (Grima *et al.* 1998).

It appears that the increase in CBF produced by infusion of K^+ is also mediated by NO. Topical superfusion of 20 mM K^+ in artificial CSF induces an elevation in rCBF which is abolished when a NOS inhibitor is added to the perfusion medium (Dreier *et al.* 1995).

These results suggest that NO may be the agent responsible for the increase in rCBF during neuronal activation. The parallel fibre system in the cerebellar vermis of anaesthetized rats has been used to test this hypothesis. Electrical stimulation of the parallel fibres produces an increase in local blood flow; this is reduced by the NOS inhibitor L-NNA, as well as methylene blue, which eliminates NO and inhibits cGMP-mediated responses (Iadecola *et al.* 1995). The reduction of rCBF is around 50% and never complete. Measurement of blood vessel diameter shows that increases in cerebellar blood flow are associated with increases in vascular diameter in the activated area. The vasodilation is greatest in smaller arterioles; however, parallel fibre stimulation also increases the vascular diameter in larger arterioles located in adjacent folia. These changes occur in areas outside the increase in neuronal activity as no field potentials are recorded in the area where these upstream vessels are located (Iadecola *et al.* 1997).

These findings suggest that neuronal activation, which involves release of glutamate, activates nNOS mainly via NMDA receptors and simultaneously induces the release of arginine from glial cells upon activation of glial ionotropic non-NMDA receptors. Thus the increase in rCBF during neuronal activation depends on the concurrent occurrence of two signals: one for the activation of the enzyme and the other for the supply of substrate (Grima *et al.* 1998).

Adenosine

The fact that inhibition of NOS causes only a 50% reduction in the increase in rCBF during neuronal activation suggests the involvement of an additional mechanism. Such a mechanism has not yet been identified, but there is some evidence that adenosine may have a role. The increase in rCBF in response to electrical stimulation of cerebellar parallel fibres is blocked by the adenosine receptor antagonist 8-sulphophenyltheophylline (8-SPT). Co-application of 8-SPT and of the NOS inhibitor nitro-L-arginine (L-NA) produces an additive effect (Li & Iadecola 1994). The activation of cerebellar Purkinje cells by two afferent systems, the parallel fibres and the climbing fibres, reveals complex activity-dependent increases in CBF. Whereas the blood flow increases stimulated by both afferent pathways are partially mediated by NO, adenosine contributes to the blood flow increase in response to stimulation of the climbing fibre but not the parallel fibre input (Akgoren *et al.* 1997). The increase in cortical blood flow by rat whisker stimulation is also inhibited by both NOS inhibition and adenosine receptor antagonists. However, the finding that superfusion of a NOS inhibitor attenuates the blood flow response to application of adenosine, led to the conclusion that both adenosine and NO are involved in coupling of cerebral blood flow to neuronal activation and that there is an interaction between the vasodilator pathways of adenosine and NO (Dirnagl *et al.* 1994). Sciatic nerve stimulation increases rCBF in the somatosensory cortex but does not cause release of adenosine. Infusion of 8-SPT decreases basal cerebral blood flow but does not block the stimulated increase in rCBF (Pellerin *et al.* 1997).

EET

Recently an alternative mechanism has been proposed for the increase in rCBF during neuronal activation which also involves the action of glutamate on glial cells. In addition to ionotropic glutamate receptors astrocytes also have metabotropic glutamate receptors. The activation of these receptors leads to a release of Ca^{2+} from intracellular stores and the release of arachidonic acid from membrane lipids by activation of phospholipase C, phospholipase A2 and diacylglycerol. The enzyme cytochrome P450 is also localized in astrocytes. This enzyme catalyses the formation of epoxyeicosatrienoic acids (EETs) which are powerful vasodilators. The hypothesis is proposed that glutamate release leads to the release of EETs from the astrocytic endfeet which envelop the cerebral blood vessels. The evidence for this hypothesis is based on the inhibition of the increase in rCBF during neuronal activation by inhibitors of P450 (Harder *et al.* 1998).

ROLE OF INCREASED rCBF.

The role of the raised rCBF during neuronal activation is to augment the delivery of oxygen and glucose to cope with the greater energy demands. Oxygen is freely diffusible and therefore the rate of oxygen delivery is

equivalent to the rate of flow of oxyhaemoglobin through the brain region. The tissue concentration of oxygen estimated electrochemically is a measure of the balance between oxygen delivery and oxygen utilization. The fact that during neuronal activation the increase in rCBF measured by the hydrogen clearance technique is paralleled by a similar increase in tissue oxygen (Lowry *et al.* 1997) suggests that there is only a very small increase in oxygen consumption. This has been confirmed using both fMRI and optical imaging (Raichle 1998). Quantitative changes in oxygen consumption in rats during neuronal activation have been calculated from measurements of changes in CBF and arterio-venous differences in oxygen. These have shown a 60% increase in CBF but only a 16% increase in oxygen consumption. As these were derived from global rather than local changes, the disparity in CBF increase and oxygen consumption in the activated area of the brain is likely to be even greater (Madsen *et al.* 1998).

Uptake of glucose

Studies similar to those for the uptake of oxygen have been carried out for the uptake and utilization of glucose. Sokoloff devised a method which uses [^{14}C]deoxyglucose, an analogue of glucose, which is not metabolized by phosphohexoisomerase and is therefore trapped in the tissue either as [^{14}C]deoxyglucose-6-phosphate or as [^{14}C]deoxyglycogen. Local tissue concentrations of ^{14}C are determined by quantitative densitometric analysis of autoradiographs of brain sections. From this, using a lumped constant which includes the various kinetic parameters, a figure is calculated which has been claimed to represent the regional cerebral glucose utilization (Sokoloff *et al.* 1977). The technique takes 45 min during which a steady state is assumed and has been criticized on a number of other points (Sacks & Fleischer 1983). Furthermore, as 2-deoxyglucose (2DG) cannot enter the metabolic cycle, its use clearly can only measure uptake and not metabolism. An alternative is the use of radiolabelled glucose, provided there is full retention of the label in the brain during the experiment. A comparison of [^{14}C]glucose labelled in positions 1, 2 and 6 showed that for measurement of total glucose utilization [6-^{14}C] glucose is the labelled substrate of choice, but when measurement of energy metabolism is required use of [1-^{14}C] glucose avoids inclusion of the non-energy-yielding pentose phosphate pathway (Hawkins *et al.* 1985). Both the uptake of [^{14}C]2DG and local cerebral glucose utilization calculated from [6-^{14}C] glucose are closely correlated with rCBF measured with iodo-[^{14}C]antipyrine (Hawkins *et al.* 1985, Sokoloff 1989).

Whereas oxygen diffuses freely throughout the brain, the compartment into which glucose is taken up from the blood is more difficult to determine. With the 2DG method radioactivity was found to be high in the neurophil, which contains the synaptic complexes (Sokoloff 1989). This was initially taken to mean that glucose was taken up into nerve terminals, which have a high energy requirement. Tsacopoulos using a preparation of retinal photoreceptors and Müller cells, showed that glucose in this preparation is taken up by the glial cells, which then export both lactate and alanine, which are subsequently taken up into the photoreceptors and oxidized (Poitry Yamate & Tsacopoulos 1991, Poitry Yamate *et al.* 1995).

Under basal conditions there is close coupling between rCBF and oxygen utilization and the energy requirements of the brain are thought to be provided by the oxidation of glucose resulting in an oxygen/glucose uptake ration of 6:1 (Madsen *et al.* 1998). Fox and Raichle in 1986 first reported a regional uncoupling of CBF and oxygen utilization during neuronal activation, induced by somatosensory stimulation in human subjects using PET (Fox & Raichle 1986). This was confirmed in a study of human visual cortex during visual stimulation, where glucose utilization and CBF increased by 51 and 50% respectively, whereas oxygen utilization increased by only 5%. (Fox *et al.* 1988). A similar disparity of oxygen and glucose uptake during neuronal activation was found in the rat (Madsen *et al.* 1998). The decrease in the ratio of oxygen/glucose uptake during activation was less in the rat, where global arterio-venous differences were measured, than in the human study using PET, where local changes were monitored.

The use of radiolabelled glucose or its analogue 2DG, cannot identify the compartment which glucose enters, when taken up from the blood. With microdialysis and implanted sensors, changes in the extracellular compartment of the brain are monitored. In microdialysis experiments in the rat striatum, stimuli which produce a motor response were found to cause a decrease in dialysate glucose during the period when rCBF was increased (Fray *et al.* 1996). Implanted oxygen electrodes and glucose sensors show an even more striking dissociation of the changes in extracellular oxygen and glucose: their concentrations in the extracellular compartment are found to move in opposite directions during the increase in rCBF which accompanies neuronal activation (Lowry & Fillenz 1997). The implication of these findings is that during the increase in rCBF the supply of oxygen greatly exceeds its utilization, while little of the glucose taken up enters the extracellular compartment directly.

In an effort to examine the effect of a reduction in neuronal activation on rCBF, and oxygen and glucose uptake, rats were given systemic administration of anaesthetics. These experiments showed a dissociation

between the changes in rCBF, tissue oxygen and glucose concentration in the extracellular compartment: the extracellular glucose concentration was reduced in all cases but the rCBF and oxygen concentration decreased with some anaesthetics and increased with others (Fillenz & Lowry 1998a and unpublished). This lends further support to the hypothesis that in contrast to oxygen, there is no simple relationship between CBF and extracellular concentration of glucose.

Glucose that enters the brain, in addition to being oxidized to CO_2 and H_2O, can be converted to lactate, to intermediates of the Krebs cycle, to amino acids or to glycogen.

Glycogen

Glycogen in the brain is localized to astrocytes. The fact that in the presence of normal levels of blood glucose it has a rapid turnover suggests that it plays a role in normal brain metabolism rather than serving as an energy store to be used when glucose supply fails. The finding that sensory stimulation causes a breakdown of glycogen (Swanson *et al.* 1992), whereas during anaesthesia and hibernation glycogen stores in the brain increase (Swanson 1992), provides evidence for the coupling of astrocytic glycogen metabolism to neuronal activity.

In astrocytic cultures various neurotransmitters, including peptides, were found to produce breakdown of glycogen (Cambray Deakin *et al.* 1988, Subbarao & Hertz 1990, Magistretti *et al.* 1993, Zhang *et al.* 1993). The effector mechanisms of the activated receptors was either a rise in cAMP or $[Ca^{2+}]_i$. Among the neurotransmitters producing glycogen breakdown is noradrenaline acting on β-adrenoceptors (Sorg & Magistretti 1991, Hertz & Peng 1992). Furthermore there is evidence that β-adrenoceptors are localized mainly on astrocytes (McCarthy 1988, Stone & Ariano 1989, Aoki & Pickel 1992). Although Stone was the first to suggest that the function of the noradrenergic action on glia may be the release of glucose for neuronal energy production (Stone & Ariano 1989), in cultured astrocytes glycogenolysis always results in the release of lactate not glucose (Dringen *et al.* 1993).

There is extensive evidence that neuronal activation leads to an increase in lactate in the brain (Fox *et al.* 1988, Fray *et al.* 1996) and that lactate can be used as a metabolic substrate by neurones (Schurr *et al.* 1988, 1997a, b). However, the stimulated release of lactate both in cultured astrocytes (Pellerin & Magistretti 1994) and *in vivo* (Fray *et al.* 1996, Demestre *et al.* 1997) appears to be dependent on glutamate uptake as it is blocked by glutamate uptake blockers.

THE ROLE OF NORADRENALINE

Evidence from a number of sources suggests that noradrenaline has a role in brain metabolism. A unilateral lesion of the locus coeruleus removes the noradrenergic innervation of the cerebral cortex on the lesioned side. Electrical stimulation results in a much reduced increase in cerebral oxidative metabolism on the lesioned compared with the control side (Harik *et al.* 1979, LaManna *et al.* 1981). Furthermore while such a lesion has no effect on the concentration of cAMP and glycogen in the unstimulated brain, during seizures and ischaemia there is a decrease in glycogen and an increase in cAMP on the unlesioned but not on the lesioned side (Harik *et al.* 1982). A more direct demonstration of the link between neuronal activity and glycogen turnover was the finding that tactile stimulation of the rat face and vibrissae accelerates the utilization of labelled glycogen in brain regions known to receive sensory input from face and vibrissae (Swanson *et al.* 1992).

In microdialysis experiments infusion of both the β-adrenoceptor agonist isoprenaline and cAMP causes an increase in dialysate glucose. In response to physiological neuronal activation the decrease in glucose which accompanies the increase in rCBF during the stimulation is followed by a delayed increase in glucose. This delayed increase in glucose is blocked by the local infusion of the β-adrenoceptor antagonist propranolol (Fray *et al.* 1996). As adrenergic receptors are found on cerebral microvessels (Harik *et al.* 1981), these effects could be mediated by effects on the cerebral circulation. However, the increase in glucose occurs after the rCBF has returned to the control value. More significantly local infusion of propranolol has no effect on the increase in rCBF that accompanies neuronal activation (Fray *et al.* 1996).

It is important to emphasize that dialysate glucose, which reflects changes in extracellular glucose concentration, represents the balance between supply and utilisation. The extracellular glucose concentration during basal conditions is 350 μM (Fray *et al.* 1997, Lowry *et al.* 1998b). Local infusion of veratridine, which opens Na^+ channels and so stimulates Na^+/K^+ ATPase and energy consumption, is accompanied by a dramatic decrease in extracellular glucose (Fray *et al.* 1997). Extracellular glucose is also reduced during hypoxia (Lowry *et al.* 1998a) and, as mentioned above, during neuronal activation (Lowry & Fillenz 1997) and in response to anaesthetics (Fillenz & Lowry 1998a). Extracellular glucose is *increased* following physiological stimulation (Fray *et al.* 1996, Lowry & Fillenz 1997) and during hyperoxia (Lowry *et al.* 1998a). The fact that local infusion of propranolol blocks some increases but not others suggests that it may serve to distinguish

between a rise caused by increased release and one by decreased utilisation. Propranolol blocks the increase in glucose following neuronal activation but not that accompanying hyperoxia (Fig. 1); this suggests that the former represents a noradrenaline-dependent increase in delivery of glucose while the latter is due to a noradrenaline-independent decrease in utilization (Fillenz & Lowry 1998b).

Proposed hypothesis

In conclusion Fig. 2 represents the hypothesis put forward in this paper. It is proposed that astrocytes play a crucial role both in the increase in rCBF and the increased supply of glucose during neuronal activation. Different receptors on astrocytes, activated by different neurotransmitters, are responsible for these two functions. Stimulation of astrocytic ampa/kainate receptors

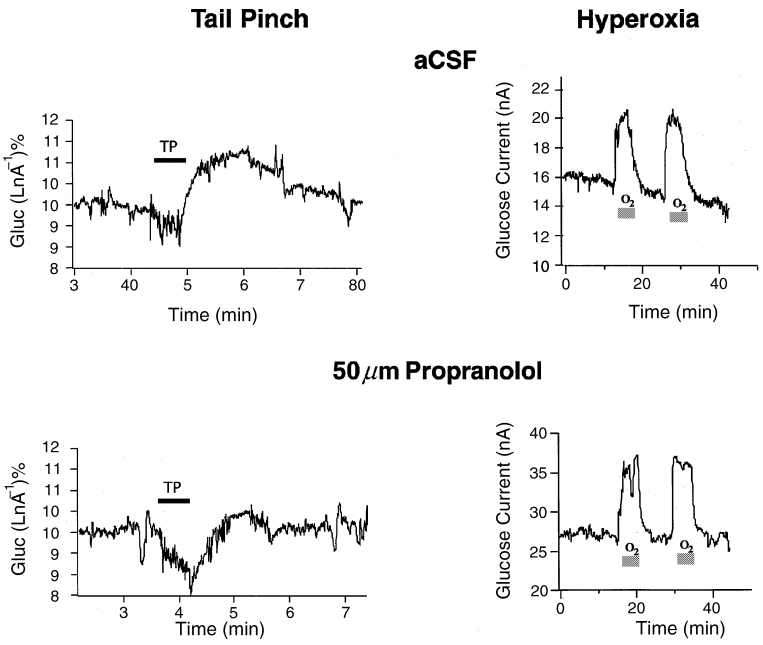

Figure 1 Changes in extracellular glucose measured with an enzyme-based sensor implanted in the rat striatum. The application of a 5 min tail pinch produces a decrease followed by an increase in glucose; exposure to a 3 min oxygen/air mixture causes an increase in glucose. Local infusion of 50 μM propranolol, a β-adrenoceptor antagonist, abolishes the increase in glucose following tail pinch but has no effect on the increase owing to hyperoxia. For futher details see Fillenz & Lowry (1998a, b).

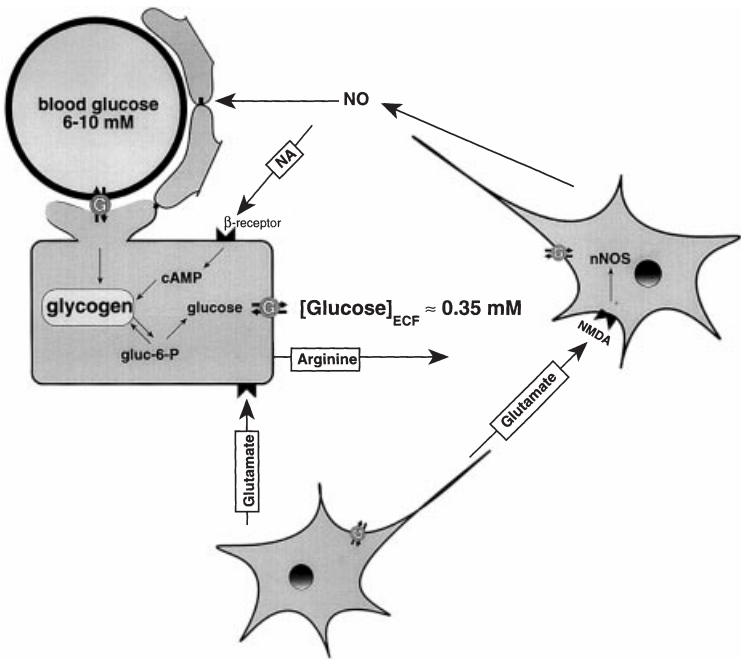

Figure 2 A schematic representation of the role of astrocytes in the activity-dependent increase in rCBF and glucose.

by glutamate leads to the release of arginine for the synthesis of NO, which is responsible for the increase in rCBF. The increase in glucose delivery by the blood, that accompanies the increase in rCBF, is taken up by atsrocytes, where some of it is converted to glycogen. Noradrenaline, acting on astrocytic β-adrenoceptors, causes the breakdown of glycogen to glucose, which enters the extracellular compartment, from where it is taken up by neurones.

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