Neuroprotective role of monocarboxylate transport during glucose deprivation in slice cultures of rat hippocampus

H. L. Cater, C. D. Benham and L. E. Sundstrom

J. Physiol. 2001;531;459-466

Neuroprotective role of monocarboxylate transport during glucose deprivation in slice cultures of rat hippocampus

H. L. Cater, C. D. Benham* and L. E. Sundstrom

*Centre for Neurosciences, Southampton General Hospital, Tremona Road, Southampton SO16 6YD and *Neuroscience Research, SmithKline Beecham Pharmaceuticals, NFSP (North), Third Avenue, Harlow, Essex CM19 5AW, UK*

(Received 16 May 2000; accepted after revision 31 October 2000)

1. The effects of energy substrate removal and metabolic pathway block have been examined on neuronal and glial survival in organotypic slice cultures of rat hippocampus.

2. Slice cultures resisted 24 h of exogenous energy substrate deprivation. Application of 0·5 mM α-cyano-4-hydroxycinnamate (4-CIN) for 24 h resulted in specific damage to neuronal cell layers, which could be reversed by co-application of 5 mM lactate.

3. Addition of 10 mM 2-deoxyglucose in the absence of exogenous energy supply produced widespread cell death throughout the slice. This was partly reversed by co-application of 5 mM lactate.

4. These effects of metabolic blockade on cell survival were qualitatively similar to the effects on population spikes recorded in the CA1 cell layer following 60 min application of these agents.

5. The data suggest that monocarboxylate trafficking from glia to neurons is an essential route for supply of energy substrates to neurons particularly when exogenous energy supply is restricted.

Glucose is the preferred energy substrate for the adult brain. The blood–brain barrier (BBB) restricts the uptake of other plentiful potential substrates such as monocarboxylates (reviewed by Cremer, 1982), although recent evidence suggests that the permeability of the BBB to lactate is higher than previously estimated (Hassel & Brathe, 2000). In healthy individuals, plasma glucose is critically regulated to ensure adequate supplies to the CNS and other tissues. While the BBB restricts access to energy supplies other than glucose, the concept has developed that astroglia and neurons compose a functional energy supply unit, with most glucose utilisation occurring in the astroglia. Glia then export lactate as an energy source that can be utilised by neurons. Thus, once within the compartment defined by the blood–brain barrier the major energy currency for use by neurons is postulated to be lactate. This process has been termed the lactate shuttle and is particularly important during periods of energy demand (Tsacoupolos & Magistretti, 1996).

When normal physiological maintenance of blood glucose fails, as in patients with insulin-dependent diabetes mellitus who suffer an insulin overdose, severe hypoglycaemia leads to loss of conciousness and, rarely, coma and death. In addition, there is now increasing evidence that, repeated severe hypoglycaemic episodes, although apparently resolving with full recovery, do correlate with long term cognitive decline and loss of hippocampal volume as determined by magnetic resonance imaging (Perros *et al.* 1997). In addition to acute interruptions in glucose supply to the whole brain, at the cellular level impairment in glucose transport with ageing may result in slow neurodegeneration (Mark *et al.* 1997). Thus there is considerable interest in understanding the cellular mechanisms essential for energy utilisation in the adult CNS.

A number of functional studies have been performed on acute preparations of cortical and hippocampal slices to substantiate the lactate shuttle hypothesis. Acute cortical slice experiments have shown that lactate can replace glucose to maintain synaptic activity (Schurr *et al.* 1988) and that lactate supplies the increased energy demands that result from activation of excitatory glutamatergic pathways (Schurr *et al.* 1999). Further acute experiments have demonstrated a role for lactate in pathophysiological protocols. Lactate, rather than glucose, fuels the recovery of function from hypoxia (Schurr *et al.* 1997*a*) and glia are the source of lactate (Schurr *et al.* 1997*b*). Izumi *et al.* (1997) used metabolic pathway inhibitors to provide further support for an important role of lactate and other monocarboxylates in supporting synaptic transmission in conditions of glucose deprivation.

Substrate utilisation by the brain during development shows important differences. Suckling neonates are more able to utilise circulating monocarboxylates, such as lactate, in part due to upregulation of expression of monocarboxylate transporters in cerebrovascular endothelia immediately postnatal (Pellerin *et al.* 1998). However, relatively little is

known of the functional expression of substrate utilisation pathways in the brain parenchyma during development and their physiological and pathophysiological importance. In infants, hypoglycaemia occurs as a result of a number of conditions of which the most common is persistent hyperinsulinaemic hypoglycaemia of infancy (PHHI), with an incidence of about 1:2500 newborns in Caucasian populations (reviewed by Aynsley-Green *et al.* 1998). Post mortem analysis of human infants and animal studies show characteristic patterns of neuronal cell death that differ from that resulting from hypoxia or ischaemia. For example, in the hippocampus, the dentate gyrus is much more sensitive than the CA1 and CA3 pyramidal cell layers to hypoglycaemic insults (Auer & Siesjo, 1988).

We have used organotypic hippocampal slices to examine the role of energy substrates on neuronal survival after 24 h of hypoglycaemia. Our aim was to investigate the contributions of the lactate shuttle to energy supply in the various neuronal cell layers of the hippocampus that differentially express monocarboxylate transporters (MCTs). In addition, we wanted to examine the importance of the lactate shuttle in a neonatal culture system, which might differ from adult tissue. The slice cultures are resistant to damage by short periods of glucose deprivation unless lactate transport is pharmacologically blocked, when pyramidal and granule cell death results. Our results suggest that the provision of lactate by glia as a neuronal energy substrate is an important facet of energy utilisation.

METHODS

Organotypic hippocampal slice cultures were prepared as described by Stoppini *et al.* (1991). Neonatal Wistar rats (8–10 days old) were killed by decapitation, the hippocampus was rapidly dissected, and transverse sections (400 μm) were cut on a McIlwain tissue chopper and immediately placed into ice-cold Geys balanced salt solution (GBSS; Gibco, UK) supplemented with 5 mg ml^{-1} glucose. Slices were plated onto sterile, semiporous tissue culture inserts (Millicell-CM, Millipore, UK, four slices per well) and maintained in culture at 37 °C in a 5% CO_2 atmosphere. The support medium consisted of 50% minimum essential medium (MEM, Gibco, UK), 25% Hanks' balanced salt solution (HBSS; Gibco, UK) and 25% heat-inactivated horse serum (ICN, UK) supplemented with 5 mg ml^{-1} glucose and 1 mM glutamine (Sigma). The medium was changed every 3 days and cultures were used for experiments after 14 days *in vitro*.

Slice cultures were then transferred into serum free medium (SF: 100% MEM, 1 mM glutamine, 5 mg ml^{-1} glucose) containing 5 μg ml^{-1} propidium iodide (PI; Molecular Probes, USA) for 20 min and then imaged under a fluoroescence microscope fitted with a rhodamine filter. Any cultures exhibiting fluorescence at this stage were discarded from further study. Following this, cultures were placed into SF medium with 5 μg ml^{-1} PI and containing either no substrate (GFM: glucose-free medium); energy substrate (30 mM glucose, 5 mM lactate); metabolic inhibitor (10 mM 2-deoxyglucose, 0·25–5 mM 4-CIN, 10–70 μM quercetin); or a combination of metabolic inhibitor and substrate. After 24 h incubation under appropriate experimental conditions, cultures were imaged for PI fluorescence and then fixed in 4% paraformaldehyde and stained with thionine. PI fluorescence was excited between 515 and 560 nm using a mercury vapour lamp and observed using a standard Leica inverted microscope fitted with a rhodamine filter. Images were captured using a monochrome CCD video camera and frame grabber, stored on either magnetic optical discs or on compact disc, and were later analysed using NIH Image 1.55 software. Quantification of cell damage was determined as previously described by Pringle *et al.* (1996) and results are expressed as the percentage area of each hippocampal subfield in which fluorescence was observed. Results are expressed as means ± S.E.M. Significance was determined by one way ANOVA followed by Dunnett's post-test.

Stocks (100 mM) of lactate, glucose, pyruvate and 2-deoxyglucose (Sigma) were all made up in GF medium to give either a 1 M or 100 mM stock so that the substrate was added to the medium to give no more than 1:10 dilution. α-Cyano-4-hydroxycinnamate (4-CIN) (Sigma, UK) was initially dissolved in heated methanol to give a 100 mM stock solution. This was then further diluted to either 1:5 or 1:10 in GF medium to give a stock for each experiment and then added to the medium in the wells at no more than 1:4 dilution. The use of methanol was controlled for by using methanol alone at the relevant dilutions in the medium and after the cultures were exposed to it for 24 h. Quercetin (Sigma, UK) was dissolved in 1 M NaOH to give a 100 mM stock and then further diluted in GF medium to give a 1 mM stock and a 10 mM stock. Quercetin was then added to the culture medium at no more than a 1:10 dilution. The pH of all solutions following changes in composition remained in the range pH 7·2 to 7·4.

To study the effects of lactate and metabolic inhibitors on synaptic function in organotypic hippocampal slice cultures, we recorded field potentials from the CA1 region in response to afferent stimulation of the Schaffer collateral commissural/associational pathway. The membrane of the Millicell culture insert on which the cultures were grown was carefully cut away from its support ring and placed in a Peltier device-controlled microscope stage incubator (Medical Systems Corp., USA) and cultures were perfused continuously with artificial cerebro-spinal fluid (ACSF; mM): NaCl 117·8, glucose 10, $MgSO_4$ 1, $CaCl_2$ 2·5, KCl 3·3, KH_2PO_4 1·26, NaH_2PO_4 26 , pH 7·4, 306 mosmol l^{-1} maintained at 34 ± 0·5°C and oxygenated by bubbling through with 95% O_2–5% CO_2. A stimulating electrode consisting of a pair of 0·28 mm twisted polyamide-insulated steel wire (Plastics One, USA) was placed into stratum lucidum at the transition between CA3 and CA1 to stimulate the afferent fibres into the CA1 region. Glass micro-recording electrodes were pulled on a standard electrode puller to a tip resistance of 5 MΩ when filled with 2·5 M NaCl. Recording electrodes were placed under visual guidance into the stratum pyramidale of the CA1 region. Single or paired 0·1 ms monopolar stimuli 0–20 V were delivered to the slice cultures at an interval of no less than 10 s. Field potential population responses were digitised (Axon Instruments, USA), recorded, stored and analysed using software developed in-house (A2D, E. Stockley, Southampton University). Population spike amplitudes and field EPSP rise times were measured in response to increasing afferent stimulus intensity (stimulus–response relationship) and a measure of recurrent inhibition was also determined by delivering paired stimuli (15 ms apart). Cultures that did not display paired-pulse inhibition or which had population spikes of less than 2 mV at a stimulus of 20 V were discarded from the study.

To study the effects of substrate modulation or metabolic inhibition on synaptic function, paired stimuli of equal intensity were delivered to the slice cultures at an intensity which was set at just

below the intensity required to produce a maximal response as determined from the stimulus–response curve (typically 10 V). Experiments were started at $t = -15$ min to determine and record a stable baseline to be established before starting the experimental procedure at $t = 0$. At this point, the perfusion was switched from the control glucose-containing ACSF to the appropriate experimental condition. Responses at the appropriate stimulus intensity were obtained every 5 min throughout the course of the experiment. At the end of the insult period, the perfusion was switched back to glucose-containing ACSF and the recovery of field potentials was monitored for a further 30 min.

RESULTS

Our initial experiments focused on determining the sensitivity of the slice cultures to interruption of supply of energy generating substrate. Glucose deprivation for 24 h was not toxic (Fig. 1). This observation suggests that there are significant energy stores in these cultures that are utilised in the absence of exogenous substrates. As neurons do not store significant energy substrates this implies that energy must be supplied by astrocytes and other satellite cells present in the culture. We investigated the role of monocarboxylate (principally lactate and pyruvate) transport by blocking MCTs with the selective inhibitor 4-CIN. 4-CIN treatment (0·5 mM) for 24 h resulted in a significant level of damage in CA1, CA3 and DG regions of the cultures. 4-CIN treatment in the presence of exogenous glucose had no effect indicating that this concentration of 4-CIN was not acting as a non-specific cytotoxin, nor was it primarily acting as a mitochondrial pyruvate transport blocker (Halestrap & Denton, 1974; Halestrap, 1975) (Fig. 1). As 4-CIN is a reversible, competitive, substrate site directed inhibitor of MCT we next checked that exogenous lactate could compete with the inhibitor. Co-application of 5 mM lactate with 0·5 mM 4-CIN resulted in less damage than 4-CIN alone, as expected if 4-CIN can be competitively displaced. Damage in CA3 was reduced almost to the unmeasurable level seen in the absence of 4-CIN (Fig. 1). Lactate replacement alone did not cause any damage (data not shown), and thus increasing exogenous lactate is not toxic *per se*.

We next looked at the effects of varying concentrations of 4-CIN on the amount of damage at 24 h in the absence of glucose (Fig. 2). The data show a concentration-dependent increase in damage at 24 h, with the CA1 and dentate granule cell layers showing greater sensitivity to 4-CIN than the CA3 region. Damage became maximal at 2·5–5 mM. This shows that 0·5 mM 4-CIN has a submaximal effect which supports the explanation that partial reversal of the 4-CIN effect by lactate was due to substrate competition. Attempts to produce similar damage with quercetin, 5-fold more potent than 4-CIN on MCT2 (Broer *et al.* 1999) were confounded by quercetin auto-fluorescence and anti-oxidant effects in the same concentration range (Gao *et al.* 2000) that might be protective. Quercetin (70 μM) induced modest damage in CA1 (11·7 $\pm$ 4·8 % ; $n = 24$, $P > 0{\cdot}05$, relative to control).

The resistance of these cultures to glucose deprivation suggests they have significant metabolisable energy stores.

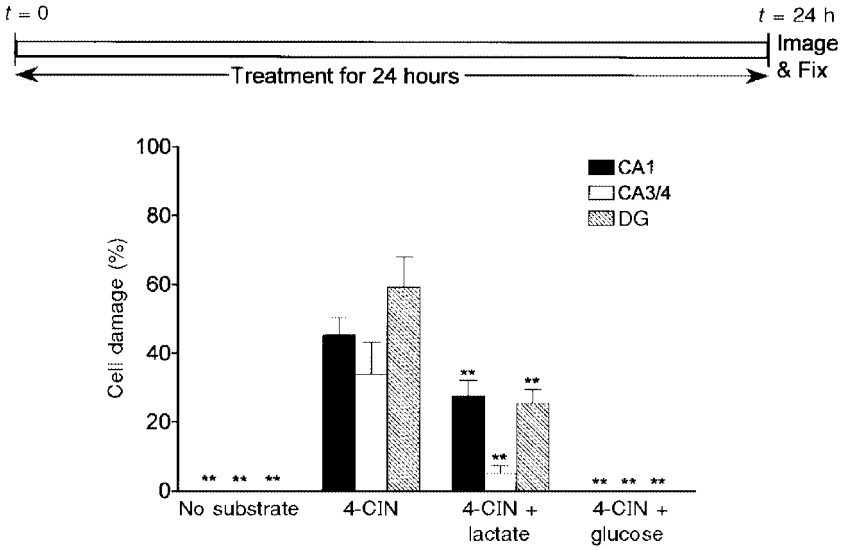

Figure 1. 4-CIN is toxic in the absence of substrate

Neuronal viability is maintained for 24 h in the absence of exogenous energy substrate but addition of the monocarboxylate transport inhibitor 4-CIN is toxic in the absence of substrate. 4-CIN shows no non-specific toxicity in the presence of glucose. All treatments were for 24 h. ■, CA1; □, CA3; and ▨, dentate granule cell regions of the cultures. Data bars are means ± S.E.M.: no exogenous substrate, $n = 24$; 0·5 mM 4-CIN in the absence of substrate, $n = 24$; 0·5 mM 4-CIN + 5 mM lactate, $n = 23$; 0·5 mM 4-CIN + 30 mM glucose, $n = 22$. Significance was tested against damage induced by 0·5 mM 4-CIN. ** $P < 0{\cdot}05$.

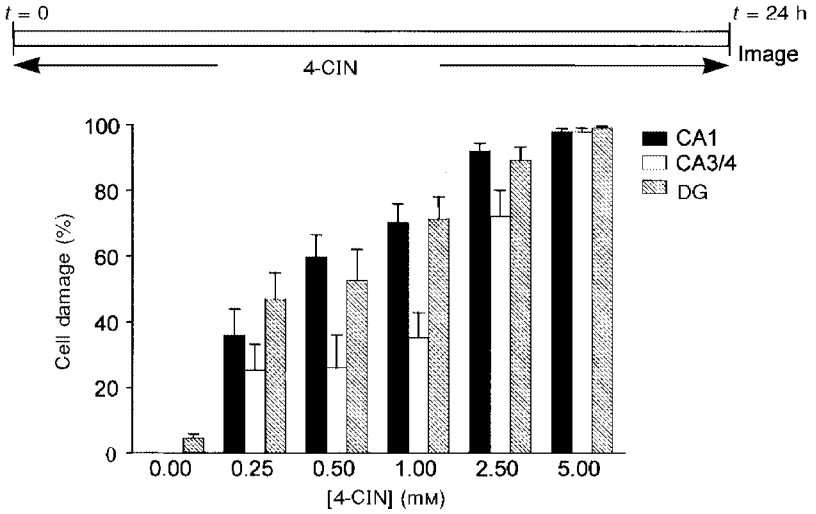

Figure 2. Concentration–response curve for damage induced by 4-CIN treatment
Cultures were exposed to 4-CIN for 24 h in the absence of exogenous energy substrate. Data bars are means ± S.E.M.: 0 mM 4-CIN, $n = 16$; 0·25 mM, $n = 24$; 0·5 mM, $n = 14$; 1 mM, $n = 24$; 2·5 mM, $n = 22$; 5 mM, $n = 23$.

We tested the role of glycolysis in supplying this energy by blocking glycolysis with 10 mM 2-deoxyglucose in the absence of glucose. This treatment resulted in maximal damage in all neuronal regions indicating an important role for glycolysis in maintaining neuronal viability in the absence of simple substrates. Addition of exogenous lactate should circumvent the glycolytic blockade by 2-deoxyglucose by supplying substrate downstream of the block. Addition of 5 mM lactate with 2-deoxyglucose did protect significantly compared with 2-deoxyglucose alone and this protective effect of 2-deoxyglucose was prevented by addition of 4-CIN (Fig. 3).

The ability to image the whole slice culture enabled us to further examine the regional specificity of the various insults. Control serum free cultures and cultures exposed to 0·5 mM 4-CIN in the presence of glucose showed no significant PI signal in any region of the slice (Fig. 4*A*). Images taken from slice cultures exposed to 10 mM 2-deoxyglucose in glucose-free medium for 24 h show that a PI signal was evident in all

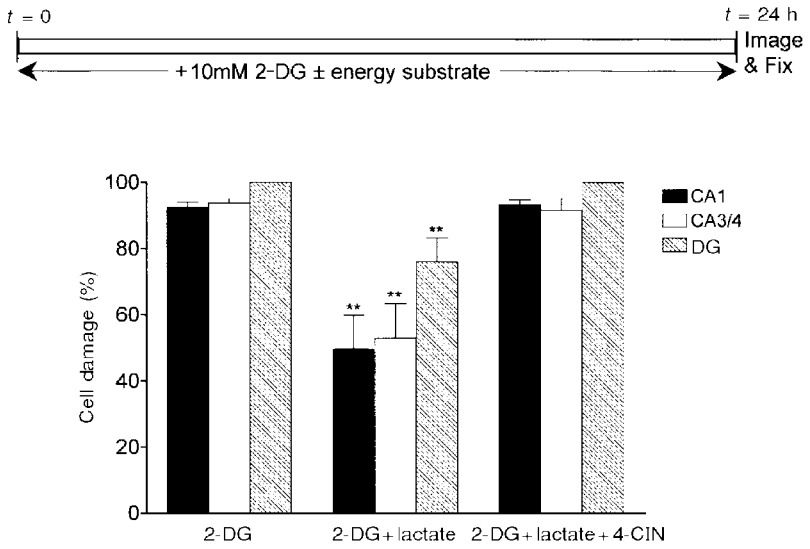

Figure 3. The effect of blockade of glycolysis with 2-deoxyglucose (2-DG)
2-Deoxyglucose, 10 mM, added in the absence of energy substrate resulted in maximal damage in all neuronal regions. Toxicity was reduced with the addition of lactate and the protective effect of lactate was reversed with 4-CIN. Data bars are means ± S.E.M.: 10 mM 2-DG, $n = 24$; 10 mM 2-DG + 5 mM lactate, $n = 22$; 10 mM 2-DG + 5 mM lactate + 0·5 mM 4-CIN, $n = 24$. Significance was tested *vs.* 10 mM 2-DG alone in glucose-free medium; $^{**}P < 0{\cdot}05$.

regions of the culture and not limited to the neuronal cell layers (Fig. 4*B*). In contrast, the damage evoked by 0·5 mM 4-CIN incubation was confined to the CA1, CA3 and dentate granule neuronal cell layers (Fig. 4*C*).

The substrate dependence of synaptic function has been explored in acute slice preparations (Izumi *et al.* 1997) but not in these organotypic cultures, so we were interested to see the acute effects of substrate manipulation on synaptic function. We recorded population spikes in the CA1 region following Schaffer collateral pathway stimulation in slice cultures exposed for 60 min to substrate changes and/or inhibitor (Fig. 5). As expected from the cell viability measures, glucose removal for 1 h had little effect, and replacement with 5 mM lactate completely prevented the trend to run-down of population spike amplitude seen in the absence of glucose. Both 2-deoxyglucose application in the absence of glucose and 4-CIN application in the absence of glucose caused a depression in population spike amplitude that reached greater than 50% inhibition after 60 min. As with the cell viability measures at 24 h, co-application of lactate was able to partially reverse the effects of 4-CIN (Fig. 5).

DISCUSSION

The results presented here can be accommodated by the basic model of metabolic substrate supply interactions in neuroglia proposed by Tsacopoulos & Magistretti (1996). This is outlined in Fig. 6 and emphasises the major role of lactate derived from glial glycogen and glucose as a neuronal energy source. Although, the earliest experimental support was obtained using the honey bee retina as a model where lactate is not the exchange currency (Tsacopoulos *et al.* 1988), subsequently, supporting data have been generated in mammalian CNS tissue (Schurr *et al.* 1988; Izumi *et al.* 1997). Our data clearly show that inhibition of the monocarboxylate transporter is toxic to neurons in the absence of exogenous energy substrates when energy must be supplied from stores. This toxicity can be prevented either by lactate or glucose addition (Fig. 1). This is consistent with the proposal that firstly, energy derived from energy stores is supplied to neurons via monocarboxylate transport as shown in pathway (3) in Fig. 6 and that this pathway may be the most significant route of energy transfer from these stores. Secondly, exogenous glucose is directly available as a substrate to glia and possibly neurons. The fact that glucose can rescue from MCT block might be explained two ways. Glucose could be directly taken up by neurons. Alternatively, it could be transported into glia and processed into lactate in sufficient concentration to overcome MCT block. As Fig. 2 shows that 0·5 mM 4-CIN is submaximal, any lactate production by glia in addition to glycogen store-derived lactate should immediately start to reverse the block. Finally, glucose transfer from glia to neurons (2) is not significant as otherwise MCT block, in the absence of added energy substrates, would not be toxic.

Exogenous lactate rescues neurons from 4-CIN toxicity (Fig. 1). This could be due to straight competition on the transporter or it could mean that 4-CIN is more potent on the glial MCT exporting lactate and is less effective at preventing exogenous uptake into neurons through the neuronal MCT. If the latter were the case, then exogenous lactate would effectively bypass the site of blockade (Fig. 6). This would require expression of two different MCT

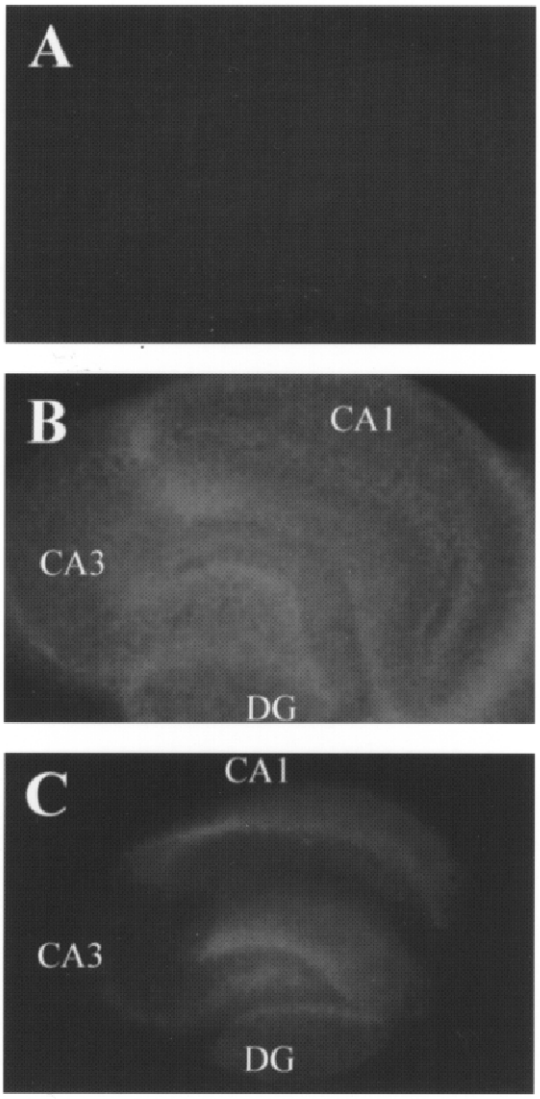

Figure 4. Damage induced by glycolytic and monocarboxylate inhibition is differentially localised
Fluorescence images of organotypic hippocampal slice cultures taken after 24 h exposed to control, serum free, showing no PI damage (*A*); and 10 mM 2-DG in the absence of exogenous energy substrate (*B*). PI signal was seen in all regions of the culture indicating damage to both glial and neuronal cell populations. *C*, 0·5 mM 4-CIN in the absence of exogenous energy substrate. The damage evoked by 4-CIN incubation was confined to the CA1, and dentate granule neuronal cell layers. The remainder of the culture showed little fluorescence, indicating minimal damage to glia.

isoforms in the appropriate locations. Of the eight known MCTs, protein distribution patterns are published for only MCT1 and MCT2 both of which are expressed in the CNS (reviewed by Halestrap & Price, 1999). In addition MCT7 and MCT8 mRNAs are relatively abundant in the human brain (Price *et al.* 1998). Localisation data and the kinetic properties of the transporters are consistent with MCT1 being the major glial MCT. MCT2 or another isoform may be the predominant form on neurons (Halestrap & Price, 1999). *In situ* hybridisation data show strong expression of MCT2 mRNA in CA1 and DG cell body layers in the hippocampus (Pellerin *et al.* 1998). This would suit flux of lactate predominantly from glia to neurons using the higher affinity for lactate of MCT2 (Broer *et al.* 1998, 1999). 4-CIN blocked MCT2 about 20-fold more potently than MCT1 when the rat transporters were expressed in *Xenopus* oocytes. IC_{50} values were 0·024 mM for MCT2 and 0·42 mM for MCT1 with 0·05 mM lactate (Broer *et al.* 1999). Thus, in our experiments, relief of 4-CIN block of MCT1 by 5 mM lactate will be greater, but taking into account the 100-fold lower substrate concentrations used in the oocyte experiments, MCT2 block is also likely to be relieved. Thus, simple substrate competition is the most parsimonious explanation of this data rather than effects on the glial MCT. Further dissection

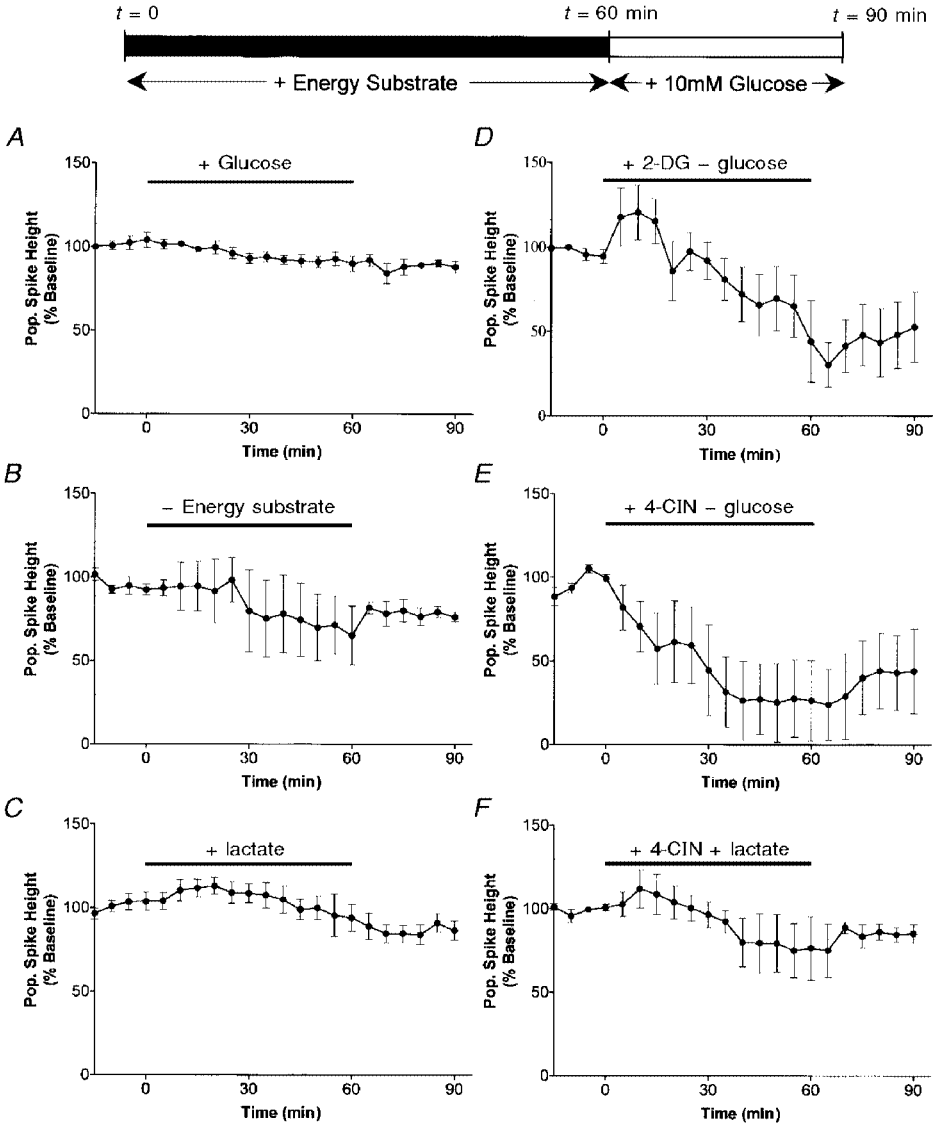

Figure 5. Acute effects of block of energy substrate supply on synaptic transmission in CA1 region of slice cultures

Addition of either 10 mM 2-deoxyglucose or 0·5 mM 4-CIN in glucose-free ACSF led to >50% decrease in the population spike height. The following substrates or blockers were added for $t = 0$–60 min; control superfusion with ACSF containing glucose (*A*), no exogenous energy substrate (*B*), 5 mM lactate (*C*), 10 mM 2-DG in the absence of energy substrate (*D*), 0·5 mM 4-CIN in the absence of energy substrate (*E*), 0·5 mM 4-CIN in the presence of 5 mM lactate (*F*). Wash out, 30 min with glucose containing ACSF followed. Means ± S.E.M. $n = 4$–5.

of the roles of different MCT isoforms will depend on more specific pharmacological blockade or gene knockout experiments.

2-Deoxyglucose would be expected to be taken up both by glia and neurons and thence to block glycolysis. This might be expected to be equally damaging to both neurons and glia (Fig. 6). Our observation of non-specific damage in the presence of 2-deoxyglucose is thus consistent with this model. This result also further emphasises the specific nature of the damage induced by MCT inhibition. In the presence of 4-CIN, lactate supply is cut off to neurons but the glia are largely unaffected. This is consistent with the energy supply in the absence of exogenous substrate originating from glycogen stores in glia which would be unaffected by this manoeuvre.

The reduced sensitivity of synaptic transmission to removal of exogenous substrate in these slice cultures is in contrast to the sensitivity of acute adult hippocampal slices (Schurr *et al.* 1988). This might reflect larger energy stores in the slices as a consequence of the time in tissue culture or the lack of maturity of the tissue, or a more flexible use of other metabolic substrates in these cultures. The dependence of neuronal viability on MCT transport, suggests that a larger glycogen store in glia or more generous glial : neuronal ratio is the most parsimonious explanation. Glial fibrillary acidic protein (GFAP) stained cultures show a high number of astrocytic processes within the culture and astrocytes can be seen to envelop the entire culture suggesting that they are present in increased numbers compared with acute slices (Pringle *et al.* 1997).

The intact brains of neonates, unlike adults, are able to utilise the rich circulating supply of lactate, pyruvate and other substrates at birth and whilst suckling (Cremer, 1982; Vicario *et al.* 1991; Nehlig & de Vasconcelos, 1993). This transient adaptation to monocarboxylate substrate supply in blood is in part achieved by a transient increase in expression of MCT1 in brain capillary endothelial cells (Pellerin *et al.* 1998). Our data suggest that the subsequent handling of substrates is very similar to that of adult brain parenchyma, with the lactate shuttle between glia and neurons playing a prominent role. The comparability of metabolic state of these slice cultures to the *in situ* properties of neonatal hippocampus has been poorly explored. Other properties examined show morphological and functional maturation at the same rate as *in situ* (reviewed by Gahwiler *et al.* 1997). Thus these cultures probably best represent the developmental status *in situ* at P15–25.

This new work highlights the role of the MCT family of transporters as key proteins in the maintenance of energy supply to neurons. Given the long term deleterious effects of metabolic stress on neurons and the likely association with neurodegenerative conditions (Mark *et al.* 1997), examination of this family of proteins might be a productive area to search for linkage with genetic diseases. The genes for MCT1 and MCT2 are localised on chromosome 1p13.2 (Garcia *et al.* 1994) and 12q13 (Lin *et al.* 1998). No specific disease linkages to these regions or to those of other known MCTs have been reported to date in the On-Line Mendelian Inheritance in Man (OMIM) database (http://www.ncbi.nlm.nih.gov/omim). Disruption at some point in this pathway may be an important source of

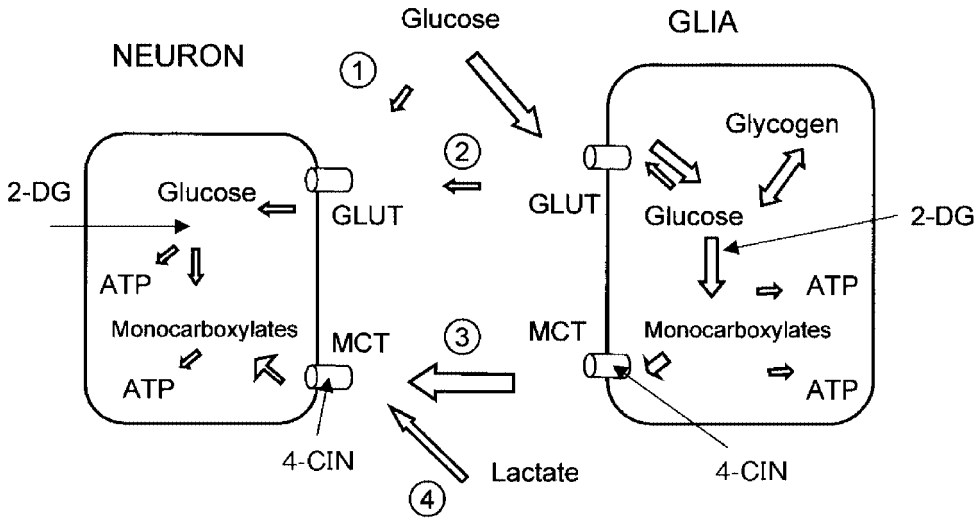

Figure 6. Energy supply to neurons and glia

Diagram showing the main sources and routes of energy supply to neurons in the slice model based on the lactate shuttle hypothesis (Tsacopoulos & Magistretti, 1996). The potential sources are (1) exogenous glucose, (2) glucose supplied from glia, (3) monocarboxylates (principally lactate) supplied from glia derived either from glycogen stores or from glucose uptake, and/or (4) exogenous lactate. 4-CIN blocks monocarboxylate transporters (MCT) in glia and neurons. 2-DG blocks glucose metabolism. Our data are consistent with pathway (3) being the major pathway.

metabolic stress leading to neuronal loss in neurodegenerative diseases.

Auer, R. N. & Siesjo, B. (1988). Biological differences between ischaemia, hypoglycaemia and epilepsy. *Annals of Neurology* **24**, 699–707.

Aynsley-Green, A., Dunne, M. J., James, R. F. L. & Lindley, K. J. (1998). Ions and genes in persistent hyperinsulinaemic hypoglycaemia of infancy; a commentary on the implications for tailoring treatment to disease pathogenesis. *Journal of Pediatric Endocrinology and Metabolism* **11**, suppl. 1, 121–129.

Bröer, S., Bröer, A., Schneider, H.-P., Stegen, C., Halestrap, A. P. & Deitmer, J. W. (1999). Characterization of the high-affinity monocarboxylate transporter MCT2 in *Xenopus laevis* oocytes. *Biochemical Journal* **341**, 529–535.

Broer, S., Schneider, H. P., Broer, A., Rahman, B., Hamprecht, B. & Deitmer, J. W. (1998). Characterization of the monocarboxylate transporter 1 expressed in *Xenopus laevis* oocytes by changes in cytosolic pH *Biochemical Journal* **333**, 167–174.

Cremer, J. E. (1982). Substrate utilization and brain development. *Journal of Cerebral Blood Flow and Metabolism.* **2**, 394–407.

Gähwiler, B. H., Capogna, M., Debanne, D., Mckinney, R. A. & Thompson, S. M. (1997). Organotypic slice cultures: a technique has come of age. *Trends in Neurosciences* **2**, 471–477.

Gao, Z., Huang, K., Yang, X. & Xu, H. (2000). Free radical scavenging and antioxidant activities of flavonoids extracted from the radix of *Scutellaria baicalensis* Georgi. *Biochimica et Biophysica Acta* **1472**, 643–650.

Garcia, C. K., Li, X., Luna, J. & Francke, U. (1994). cDNA cloning of the human monocarboxylate transporter 1 and chromosomal localization of the SLC16A1 locus to 1p13.2-p12. *Genomics* **23**, 500–503.

Halestrap, A. P. (1975). The mitochondrial pyruvate carrier. Kinetics and specificity of substrates and inhibitors. *Biochemical Journal* **148**, 85–96.

Halestrap, A. P. & Denton, R. M. (1974). Specific inhibition of pyruvate transport in rat liver mitochondria and human erythrocytes by α-cyano-4-hydroxycinnamate. *Biochemical Journal* **138**, 313–316.

Halestrap, A. P. & Price N. T. (1999). The proton linked monocarboxlate transporter (MCT) family: structure, function and regulation. *Biochemical Journal* **343**, 281–299.

Hassel B. & Brathe A. (2000). Cerebral metabolism of lactate *in vivo*: Evidence for neuronal pyruvate carboxylation. Journal of *Cerebral Blood Flow and Metabolism* **20**, 327–336.

Izumi, Y., Benz, A. M., Katsuki, H. & Zorumski, C. F. (1997). Endogenous monocarboxylates sustain hippocampal synaptic function and morphological integrity during energy deprivation. *Journal of Neuroscience* **17**, 9448–9457.

Lin, R. Y., Vera, J. C., Chaganti, R. S. K. & Golde, D. W. (1998). Human monocarboxylate transporter 2 (MCT2) is a high affinity pyruvate transporter. *Journal of Biological Chemistry* **273**, 28959–28965.

Mark, R. J., Pang, Z., Geddes, J. W., Uchida, K. & Mattson, M. P. (1997). Amyloid β-peptide impairs glucose transport in hippocampal and cortical neurons: involvement of membrane lipid peroxidation. *Journal of Neuroscience* **17**, 1046–1054.

Nehlig, A. & Pereira de Vasconcelos, A. P. (1993) Glucose and ketone body utilization by the brain of neonatal rats. *Progress in Neurobiology* **40**, 163–221.

Pellerin, L., Pellegri, G., Martin, J.-L. & Magistretti, P. J. (1998). Expression of monocarboxylate transporter mRNAs in mouse brain: Support for a distinct role of lactate as an energy substrate for the neonatal *vs.* adult brain. *Proceedings of the National Academy of Sciences of the USA* **95**, 3990–3995.

Perros, P., Deary, I. J., Sellar, R. J. & Frier, B. M. (1997). Magnetic resonance imaging and spectroscopy of the brain in IDDM patients with and without a history of severe hypoglycaemia. *Diabetes Care*, **20**, 1013–1018.

Price, N. T., Jackson, V. N. & Halestrap, A. P. (1998). Cloning and sequencing of four new mammalian monocarboxylate transporter (MCT) homologues confirms the existence of a transporter family with an ancient past. *Biochemical Journal* **329**, 321–328.

Pringle, A. K., Iannotti, F., Wilde G. J. C., Chad, J. E., Seeley, P. J. & Sundstrom, L. E. (1997). Neuroprotection by both NMDA and non-NMDA receptor antagonists in *in vitro* ischemia. *Brain Research* **755**, 36–46.

Pringle, A. K., Sim, L., Kennedy, J., Wilde, G. J. C., Benham, C. D., Iannotti, F. & Sundstrom, L. E. (1996). The selective N-Type calcium channel antagonist omega conotoxin MVIIA is neuroprotective against hypoxic neurodegeneration in organotypic hippocampal slice cultures. *Stroke* **27**, 2124–2130.

Schurr, A., Miller, J. J., Payne, R. S. & Rigor B. M. (1999). An increase in lactate output by brain tissue serves to meet the energy needs of glutamate-activated neurons. *Journal of Neuroscience* **19**, 34–39.

Schurr, A., Payne, R. S., Miller, J. J. & Rigor, B. M. (1997*a*) Brain lactate, not glucose, fuels the recovery of synaptic function from hypoxia upon re-oxygenation: an *in vitro* study. *Brain Research* **744**, 105–111.

Schurr, A., Payne, R. S., Miller, J. J. & Rigor B. M. (1997*b*). Glia are the main source of lactate utilised by neurons for recovery of function post-hypoxia. *Brain Research* **774**, 221–224.

Schurr, A., West, C. A. & Rigor, B. M. (1988). Lactate-supported synaptic function in the rat hippocampal slice preparation. *Science* **240**, 1326–1328.

Stoppini, L., Buchs, P. A. & Muller, D. (1991). A simple method for organotypic cultures of nervous tissue. *Journal of Neuroscience Methods* **37**, 173–182.

Tsacopoulos, M., Evequoz-Mercier, V., Perrottet, P. & Buchner, E. (1988). Honeybee retinal glial cells transform glucose and supply the neurons with metabolic substrate. *Proceedings of the National Academy of Sciences of the USA* **85**, 8727–8731.

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

Vicario, C., Arizmendi, C., Malloch, G., Clark, J. B. & Medina, J. M. (1991). Lactate utilization by isolated cells from early neonatal rat brain. *Journal of Neurochemistry* **57**, 1700–1707.

Acknowledgements

H.L.C. was funded by an MRC CASE Award.

Corresponding author

C. D. Benham: Department of Neuroscience Research , SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK.

Email: christopher_d_benham@sbphrd.com

Neuroprotective role of monocarboxylate transport during glucose deprivation in slice cultures of rat hippocampus

H. L. Cater, C. D. Benham and L. E. Sundstrom

J. Physiol. 2001;531;459-466