Design and development of a miniaturised flow-through measuring device for the in vivo monitoring of glucose and lactate
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Chapter 9

Turnover of extracellular glucose and lactate in the rat striatum estimated by equilibrium microdialysis and sensor technology
Summary

Intercellular trafficking of energy substrates is essential to meet cerebral energy demand. It is as yet not known which proportion of total energy metabolism is derived from the intercellular compartment. The aim of the present study was to quantify glucose and lactate trafficking in vivo through the intercellular space of the striatum of the conscious rat.

Ultraslow (equilibrium) microdialysis of the striatum of freely moving rats was used to estimate intercellular levels and the turnover rates of glucose or lactate. No or increasing amounts of glucose or lactate were infused until steady state. From the difference of the concentration between the in- and outflowing perfusate the turnover rate constants of glucose and lactate in the intercellular space is calculated. From these constants, the steady state levels and the size of the intercellular space turnover rates per g tissue were derived. Steady state levels (± SD) of glucose and lactate 2-4 days after probe implantation were 0.23± 0.12 and 0.66± 0.36 mM, respectively. The turnover rate of lactate was 0.089 ± 0.016 µMol/g/min and that of glucose 0.024 ± 0.003 µMol/g/min. These figures show that less than 10% of rat striatal energy substrates is transferred via the extracellular space and was mostly attributed to lactate trafficking. The values are close to those of the glucose-lactate shuttle estimated from a total energy balance.

9.1 Introduction

Trafficking of substrates between cells is considered essential to meet energy demand of the brain. Accordingly, it has been hypothesized that neuronal energy consumption depends, at least in part, on the release of glucose and lactate from astroglia cells and on subsequent diffusion to neurons via the brain intercellular space (ICS)1-10. The importance of an estimation of the concentration of glucose and lactate in the ICS has been recognized by several authors11-21. Concentrations ranging from 0.35 – 3.3 mM glucose11 and 0.35 – 1.1 mM lactate18-21 have been reported. There are, however, no reports on either estimates of the turnover rate of glucose and lactate of the ICS or of the proportion of energy substrates that is transferred via this compartment.
Here, we describe estimates of the steady state levels and the amounts of lactate and glucose that diffuse through the ICS of the striatum of the conscious and freely moving, rats. Our approach is based on the application of equilibrium microdialysis (eMD). With the conventional microdialysis (MD) perfusion rates over 1 µL.min⁻¹ are most often used. Such flow rates result in considerable concentration gradients of the analytes over the MD probe membrane ranging from ratios between 5 up to 20, depending, among other, on the size of the dialysis probe and the perfusion rate applied²²,²³. Therefore, rather complicated methods have to be proposed to derive the real ICS levels from dialysate measurements. A relatively simple approach is based on the assumption that the recovery \textit{in vivo} is the same as that \textit{in vitro}. In most cases, including brain investigations, such an assumption is not allowed, because \textit{in vivo} diffusion is limited as the result of the tortuosity of the tissue and, moreover, the probe may – at least in part – become occluded with tissue²⁴,²⁵. We²⁶ and others²⁷,²⁸ have proposed eMD for the quantitative estimation of concentrations of substrates in the ICS. Using perfusion rates of 100 nanoliter per minute (10⁻⁹ L.min⁻¹ = nL.min⁻¹) or less and conventional microdialysis probes, complete equilibrium between ICS and the perfusate is reached, even if more than 80% of the surface of the probe becomes covered with tissue²⁶. Moreover, we demonstrated that ultraslow ultrafiltration (collecting interstitial fluid directly through a semi-permeable membrane) and eMD resulted in identical levels of glucose and lactate²⁶. A major advantage of eMD is that the real \textit{in vivo} concentrations in the ICS are continuously measured²⁹. eMD does not only allow to monitor the real levels of glucose and lactate in the ICS of a conscious rat brain striatum, but to estimate their turnover rates in that compartment as well. We propose here a simple approach to estimate these turnover rates based on the loss of glucose or lactate added to the perfusate at steady state levels. In practice, the decrease in the concentrations of lactate or glucose in the influx and efflux were measured at a constant flow rate of 100 nL.min⁻¹. This approach is justified, because it has been shown that acute hyperglycemia does not affect glucose cerebral consumption in conscious rats³⁰,³¹. During low rate perfusion rapidly new steady state levels are achieved. The difference between the amounts infused and collected per time unit reflects the sum of diffusion and consumption of the substrates in the ICS surrounding the MD-probe. The cellular transporters of both lactate and glucose are bi-directional, so the rate of disappearance is particularly due to metabolism at steady state perfusion, rather than to transport over the cellular membrane. Because passive diffusion may also contribute to disappearance the present approach reveals maximal turnover rates of glucose or lactate in the ICS. Assuming a normal ICS compartment of 18% of tissue¹², we calculated the
maximal ICS turnover of glucose and lactate per g wet weight tissue. On-line eMD of glucose and lactate was possible because of the application of adapted micro-sensor technology, as described previously by us.\(^{31}\)

9.2 Materials and methods

9.2.1 Materials

Glucose oxidase from *Aspergillus niger* (EC 1.1.3.4, grade I, 25,000 units per 84.5 mg solid) and lactate oxidase from *Pediococcus species* (grade I, 200 units per 6.74 mg solid) were obtained from Boehringer Mannheim (Almere, Netherlands). D(+) glucose and L(+)-lactate for standard solutions and 1,3-phenylenediamine for the permselective sensor membrane was from Sigma Chemical Co. (Amstelveen, Netherlands). All other chemicals were of pro-analysis quality and were purchased from E. Merck (Amsterdam, The Netherlands). Double quartz-distilled water was used for all aqueous solutions. The composition of the carrier solution during eMD is a phosphate-buffered saline (PBS) solution (mM): NaCl (136.9), KCl (2.7), KH\(_2\)PO\(_4\) (1.5), CaCl\(_2\) (0.9), MgCl\(_2\) (0.5) and Na\(_2\)HPO\(_4\) (8.1). The pH was adjusted to 7.4 and the solution gassed with He before use. Standard solutions of glucose or lactate were prepared by diluting the stock solutions of glucose (50 mM) in PBS and were allowed to reach mutarotational equilibrium before use (24 h).

9.2.2 Probe construction

Microdialysis probes were constructed by inserting fused silica (150 µm od, 50 µm id) (Aurora Borealis Control, Assen, Netherlands) into a fiber of an artificial kidney (AN69HF, acrylonitrile and sodium methallyl sulfonate copolymer, Filtral 16; Hospal Ind., Meyzieu, France, 290 µm od, 240 µm id, molecular mass cut off 20 kDa). The tip was sealed with cyanoacrylic glue. A second fused silica tube is inserted into the fiber and served as the outlet. The fiber (total length 6 mm) as well as the inlet (total length of 8 mm) and outlet fused silica tubing (total length of 5 mm) were glued into a stainless steel cannula with cyanoacrylic glue, leaving an active length of 4 mm.
9.2.3. Surgery

Male Wistar rats (250 - 400 g, Harlan Nederland, Horst, Netherlands) were housed individually in a light-controlled room (light/dark: 0700/1900 h). Food and drink were provided ad libitum. The rats were anaesthetized by an intramuscular (i.m.) injection of 0.4 mg/kg body wt Hypnorm (Janssen, Beerse, Belgium) and an i.p. injection of 0.24 mg/kg body wt pentobarbital sodium (Sanofi, Maassluis, Netherlands) and placed in a stereotaxic frame. Using a rectal temperature probe and an infant heat pad (IGB, Germany), the body temperature was maintained between 36.5 and 37.5 °C during surgery. The microdialysis probe was implanted into the left striatum (from bregma, A +1.0 mm, L 2.5 mm; -6.0 mm for the surface of the skull) and secured with skull screws and dental acrylate. Additionally, a home-made aluminum screw was placed around the inlet and outlet of the probe and glued on the skull with dental acrylate. A home-made aluminum cap (diameter about 2 cm) could be easily screwed around the inlet and outlet of the probe for protection, that can easily be removed. Before measurements the animals were allowed to recover for 24 h. The Committee on Animal Bio-Ethics of the University of Groningen approved the here described procedures.

9.2.4 Preparation of glucose and lactate biosensor

Flow-through nanoliter biosensors were used as described earlier in detail32. Briefly, the flow-through cell was constructed by placing close to each other two platinum wires (0.10 mm diameter) and a Ag/AgCl wire (0.125 mm diameter; Drijfhout, Amsterdam, Netherlands) in a 0.005 inch id Tygon tubing (Skalar Analytical, Breda, Netherlands) by means of a 0.50 x 16 mm Luer Lock needle (B. Braun, Melsungen, Germany). Possible leakage from in the Tygon tubing was prevented with cyanoacrylic glue. Prior to immobilization of the enzyme, the flow-through cell is washed with methanol, 10% v/v hydrogen peroxide solution in water and 0.1 M phosphate buffer pH 7.0 by means of a 1 ml syringe (Becton Dickinson, Etten-Leur, Netherlands) equipped with a 0.40 x 12 mm Luer Lock needle (B. Braun, Melsungen, Germany). Biosensors were produced by filling the syringe with a solution containing 2 mg.ml⁻¹ of enzyme (approximately 500 units per ml of glucose oxidase respectively 50 units per ml of lactate oxidase) and 10 mg.ml⁻¹ (90 mM.l⁻¹) of 1,3-phenylenediamine in 0.1 M phosphate buffer pH 6.9. The syringe was placed in a model 22 syringe pump (Harvard Apparatus, Kent, UK) and the wires outside the tubing were connected with a model DECADE electrochemical detector (Antec Leyden, Leiden,
Netherlands) by means of crocodile clips. Glucose biosensors were produced by electro-polymerization at +0.8 V vs. Ag/AgCl for one hour at a flow rate of 500 nl.min\(^{-1}\) followed by an additional 30 minutes using the monomer solutions without the enzyme. Lactate biosensors were produced by electro-polymerization by sequentially potentially cycling from −0.2 to +0.1 V vs. Ag/AgCl at a scan rate of 50 mV.s\(^{-1}\) for at least 20 potential sweeps. Before storage and/or use, the biosensors were rinsed with 0.1 M phosphate buffer pH 6.9 for 30 min. In between, the biosensors were stored in the refrigerator at 4-8 °C. Lactate or glucose were measured in a discontinuous and a continuous mode, respectively.

### 9.2.5. Experimental conditions

Following surgery, the rats were individually housed in plastic animal’s cages (20x30cm) with free access to food and water. Experiments were carefully carried out to maintain sterility. On experimental days, the probe was washed with sterile physiological saline solution (0.9% NaCl) and the inlet of the probe was connected to a 1 ml syringe (Becton Dickinson, Etten-Leur, Netherlands) via polyethylene tubing (id 400 µm, od 750 µm, Skalar, Breda, Netherlands) with a length of approximately 40 cm, whereas the outlet of the probe was connected to the measuring device via fused silica tubing (150 µm od, 50 µm id) (Aurora Borealis Control, Assen, Netherlands) also with a length of approximately 40 cm, allowing the animals free movement. Microdialysis was performed with PBS buffer at a flow rate 100 nl.min\(^{-1}\) by placing the syringe in a model 22 syringe pump (Harvard Apparatus, Kent, UD). Measurements were performed on day 1, 2, 3, 4, 5 and 6 after implantation of the probe. On every day steady state concentrations of respectively glucose and lactate were measured approximately 30 minutes after connecting the rat, allowing stabilization of the signal. In this case both syringe and inlet tubing was filled with PBS buffer. Next, the turnover rate of glucose or lactate was measured by filling the syringe and inlet tubing with standard solutions in PBS buffer containing increasing amounts of glucose or lactate. For every measurement, new steady state levels of both substrates were achieved and recorded within 45 minutes of perfusion. After the experiments, the rat was disconnected, the probe was washed again with physiological salt solution and the rat was brought back to the light-controlled room.

### 9.2.6 Histology

On completion of testing, rats were killed by overdose of sodium pentobarbital. Brains
were removed and placed in 4% formalin buffered solution, pH 7.4 for a minimum of 3 days. Before sectioning, brains were dehydrated for approximately 18 hours in a 30% sucrose saline buffer pH 7.4, frozen and sliced with a Reichert-Jung cryostat at a temperature of –13 °C. Sections of 40 µm were taken through the brain areas of cannula placement. Animals showing signs of tissue damage other than cannula track, or where the probe was placed outside the striatum were discarded.

9.2.7 Calculations

The calculations are based on the model as defined in Figure 1. It is assumed that the cerebral release of lactate into the blood circulation is negligible. We assume that the properties of the tissue surrounding the probe are identical to those of the undisturbed ICS, thus of brain tissue without an implanted MD-probe. Under steady-state conditions with a probe that is perfused at low rates (= eMD) the following equation defines the amounts of lactate or glucose that is per time unit transported towards and outwards the dialysis probe. At such low rates we assume that the concentrations of glucose and lactate in the (out-flowing) dialysate is the same as that in the ICS (and at 0-rate perfusion, meaning that the diffusion over the probe membrane is not limiting), so the following equation can be written:

![Compartmental model used for the calculations.](image)

*Figure 1:* Compartmental model used for the calculations.

For definitions of the used parameters see section methods.
\[ K_1 \cdot C_{\text{brain}} = k_2 \cdot C_{\text{ss}} + F \cdot C_{\text{out}} = (k_2 + F) \cdot C_{\text{ss}}, \]  
\[ \text{[equation 1]} \]

in which,
- \( K_1 \) = a transport constant (or composition of various constants; arbitrarily defined) of brain glucose or lactate towards the probe;
- \( C_{\text{brain}} \) = apparent (both cellular and intercellular) concentration of the substrates in the brain;

So the term \( K_1 \cdot C_{\text{brain}} \) equals the amount of lactate or glucose that is released from brain tissue per time unit reaching the probe.

- \( k_2 \) = transport constant from the probe as a part of the ICS into the brain tissue;
- \( C_{\text{ss}} \) = measured concentration under steady state conditions (without added glucose or lactate to the perfusate).

So the product \( k_2 \cdot C_{\text{ss}} \) represents the amount of lactate or glucose that diffuses per time unit from the probe into brain tissue.

- \( F \) = flow rate (in the present experiments, set at 100 nl/min);
- \( C_{\text{out}} \) = \( C_{\text{ss}} \) concentration glucose or lactate measured in the dialysate (only when no glucose or lactate was added to the perfusate).

When glucose or lactate are added to the perfusate (\( C_{\text{in}} \)) and assuming that the rates of inflow and the outflow are identical and (new) steady states are reached, the amounts of the substrates transported or diffused per time unit can described as:

\[ K_1 \cdot C_{\text{brain}} + F \cdot C_{\text{in}} = (k_2 + F) \cdot C_{\text{out}}, \]  
\[ \text{[equation 2]} \]

Substituting the term \( K_1 \cdot C_{\text{brain}} \) by combining equation [1] and [2] results in:

\[ (k_2 + F) \cdot C_{\text{ss}} + F \cdot C_{\text{in}} = F \cdot C_{\text{out}} + k_2 \cdot C_{\text{out}}, \]  
that can be rewritten as:

\[ k_2 = F \frac{(C_{\text{in}} - C_{\text{out}} + C_{\text{ss}})}{(C_{\text{out}} - C_{\text{ss}})}. \]  
\[ \text{[equation 3]} \]

The constant \( k_2 \) (possibly a combination of rates of transport constants and catabolism, see discussion below) as defined here is related to the volume of the probe. The volume of the probe (\( V_{\text{prob}} \)) in the striatum is 0.48 µl.; the ICS volume is 180 µl/g of brain tissue. In the calculations it is assumed that the turnover of lactate equals \( \frac{1}{2} \) that of glucose. Based upon these assumptions, the flux of glucose or lactate per gram brain tissue (in µM/ g/ min) can thus be calculated as:

\[ \frac{146}{100} \]
Flux = (180/0.48) C_{ss} (k_2 + F).

The percentage of metabolic trafficking via the ICS (R %) is calculated as the ratio between the calculated Flux and the known Cerebral Metabolic Rate (CMR) for glucose in striatum (0.82 µM/g/min; the mean value of the values given by Barbelivien et al., Duelli et al. and Horinaka et al.) x100% as follows:

R (%) = ([Flux]/(CMR)) x100%  \hspace{0.5cm} \text{[equation 4]}

9.2.8 Presentation of the results

Values are given ± SEM. Significance of the differences was calculated with Student t test. P-values < 0.05 are considered significant. ANOVA was used to test possible significant trends of steady state levels over time following implantation and of concentration dependency of calculated ICS turnover rate.

9.3 Results

Examples of recordings are shown in Figure 2. Rat striatal steady state concentrations of ICS (C_{ss}) glucose (n = 10) and lactate (n= 6) were measured up to 6 days following the

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Typical recorder outputs during the continuous monitoring of glucose (a) and lactate (B): In vitro calibration of the biosensor with 5 mM glucose respectively 2.5 mM lactate (a), directly followed by in vivo measurements: (b) steady state concentration of glucose respectively lactate, and new steady state concentrations after the addition of 0.5 mM glucose respectively 1.5 mM lactate (c); 1 mM glucose respectively 3 mM lactate (d); 2.5 mM glucose respectively 4 mM lactate (e); and 5 mM lactate (f).}
\end{figure}
implantation of a MD probe. The time course of $C_{ss}$ is given in figure 3. The lowest levels (+ SEM) of glucose were measured at days 2, 3 and 4 and were $0.35 \pm 0.07$, $0.18 \pm 0.04$ and $0.31 \pm 0.05$ mM, respectively. The lactate levels (± SEM) did not significantly vary over 4 days; these levels were at day 2, 3 and 4, $0.64 \pm 0.03$, $0.68 \pm 0.03$ and $0.58 \pm 0.02$ mM, respectively.

At day 3 and 4 the turnover rates of glucose ($n = 6$) and lactate ($n = 6$) were estimated by infusing glucose (0.5, 1.0, 2.0, 2.5 or 5.0 mM) or lactate (1.5, 2.5, 3.0, 4.0 or 5.0 mM). On day 3 six rats were studied either with lactate (3 rats) or with glucose (3 rats); the same rats were studied on day 4, but now applying the other substrate. Based upon these measurements, the transport constant $k_2$ and $R$ (%) were calculated, using equations [3] and [4]. We calculated also the correlation between the amounts of added glucose or lactate and $k_2$ as an index of utilized substrate. There was no significant correlation between added substrate and calculated turnover rate or $R\%$ of glucose and lactate (Figure 4). From these results, the mean ratio $R$ (%) per added amount of glucose or lactate as well as the overall mean ratio $R$ (%) were estimated to be less than 10% of the total energy consumption (Figure 4 and Table 1). It appeared that less than 3% of glucose and 7% of lactate is transported via the ICS, so less than 10% of the striatal energy metabolism can be attributed to diffusion of substrates via the intercellular compartment.
Table 1:
Steady state levels, absolute and relative turnover rate of intercellular glucose and lactate measured in the striatum of freely moving rats (6 rats studied 3/4 days after implantation of the dialysis probe; M ± SD, N= 6).

| Glucose | | Lactate | | Total in Glucose equivalents |
|---------|------------------|---------|------------------|
| Steady state levels Css. | 0.23 ± 0.12 mM | Steady state levels Css. | 0.66 ± 0.36 mM | Turnover ICS | 0.068 ± 0.01 mMol/g/min |
| Turnover ICS | 0.024 ± 0.003 µMol/g/min | Turnover ICS | 0.089 ± 0.016 mMol/g/min | Relative turnover | 8.3 ± 0.7 % |
| Relative Turnover | 2.9 ± 0.4 % | Relative turnover | 5.4 ± 0.8 % |

Figure 4:
Mean estimated proportion (as % + SEM) of extracellular glucose and lactate turnover in the conscious rat as related to the total glucose consumption. Upper panels show that the estimates were independent on added glucose and lactate. Lower figure is composed from the data of individual rats at days 3 and 4 following probe implantation. Number of rats: 6.
9.4 Discussion

9.4.1 ICF glucose and lactate levels

The combination eMD with a flow-through nanoliter biosensor allowed us to monitor on a real time basis quantitatively and accurately ICS glucose or lactate levels in freely moving rats at various time intervals following probe implantation without complicated calibration procedures. Accordingly, concentrations ranging from 0.20 – 0.90 mM for glucose and 0.57 – 0.73 mM for lactate in the striatum of freely moving rats were obtained. These values are among the lowest reported (see introduction). The ICS levels of glucose decreases up to 3 days followed by an increase 4 to 6 days after implantation of the probe. These observations are comparable with those found by Fellows et al.\textsuperscript{14}, who reported a reduction of 75% of the ICS glucose content over 72 h following implantation of the probe and were attributed to a disturbances in glucose metabolism immediately after the implantation of the probe. According to them, the blood-brain-barrier is resealed within 2 h, whereas the local cerebral blood flow and glucose utilization in tissue surrounding the probe becomes normal within 24 h. Groothuis et al.\textsuperscript{38} reported, however, that the blood-brain-barrier permeability is affected biphasically: a prompt increase shortly after insertion, followed by a second increase several days following placement of the probe. They indicate that blood-brain-barrier dysfunction persists for at least 28 days after probe implantation. If this is true, the ICS glucose content may even be lower than observed here. Measurements at longer time intervals are not recommended because of gliosis, that starts in the vicinity of the probe already within 2 days after implantation\textsuperscript{39,40}. Although the blood-ICS-barrier may fluctuate over time, we never observed glucose values approaching blood levels, demonstrating that the barrier remained largely intact throughout the experiments. In contrast to glucose, no significant deviations in lactate content were observed over the 4 days after probe implantation. This observation may indicate that lactate levels are determined by properties of the bi-directional membrane transporters, thereby expanding the volume of distribution of lactate and thus suppressing local and short lasting fluctuations. Brain tissue levels of lactate may also remain relatively constant, because even under normoxic conditions lactate leaves the brain, particularly when local levels eg. in glia adjacent to the vascular bed, tend to become higher than blood plasma levels\textsuperscript{32}. 
9.4.2 About the method of turnover rate estimations

It is argued here that the turn-over values presented here are probably maximum values and may therefore be considered as over-estimations of the turnover rates at physiological steady state conditions. Basic in our model is the assumption that the rate constants of disappearance of glucose or lactate from the dialysis probe at eMD and the ICS were similar and that they were not affected by the concentration of the substrates. The concentrations of glucose and lactate applied are well below the $K_m$ values of their transporters, so it is unlikely that the rates of disappearance become limited at high concentrations. Indeed, the calculated $k_2$ values were concentration-independent, thereby suggesting that this value was also valid at steady state perfusion, thus without addition of substrates to the perfusate. With the present experimental design it is impossible to distinguish between – passive – diffusion or – active – metabolism of substrates added to the perfusate. Such a distinction would be possible, when high and carrier-saturating concentrations of lactate or glucose were applied. If diffusion is the most prominent route of disappearance of the substrate out of the probe (and ICS) the calculated $k_2$ values should be concentration-independent, even when very high amounts of glucose or lactate (e.g. at 25mM glucose or 10 mM lactate) were infused. Conversely, if glucose and lactate loss is due to carrier-mediated transport, the $k_2$ should approach a maximum value at such high substrate exposures. Direct diffusion from the probe to blood is highly unlikely, considering the large concentration gradient of glucose that has to be overcome at any of the presently applied concentrations of glucose.

According to the classical model, ICS glucose in the brain is derived directly from the bloodstream and changes in neuronal energy requirements are met by changes in the cerebral blood flow\textsuperscript{41,42}. A direct relationship between blood and ICS glucose (and lactate) is unlikely, considering the energy buffering potential of endogenous glycogen\textsuperscript{10,43} and the role of intracerebral lactate for energy metabolism. Glucose utilization within the brain is believed to be limited by phosphorylation, rather than by transport\textsuperscript{12,44}. So exposure of brain tissue to high glucose does not necessarily affect energy metabolism. Indeed, Duckrow and Bryan\textsuperscript{30} and Orzi et al.\textsuperscript{31} showed that acute hyperglycemia does not lead to altered glucose consumption. Several studies\textsuperscript{13-15} have shown that transient changes in extracellular glucose concentration occur following local neuronal activity, which is compatible with the idea that metabolism plays an important role in determining the ICS levels.

Although the applied ICS-turn-over rates may be maximum values as argued, it is as yet
uncertain whether in addition to the studied routes, alternatives and here unnoticed transfer of substrates is possible. Such alternative route could, for instance, be a direct exchange of substrates between glia and neurons in close apposition to each other, so little substrate could reach the MD probe. Alternatively, glucose and lactate may diffuse in brain tissue through close junctions, that have been observed between astroglia cells. Such close junctions, however, have never been seen between neurons and glia. Moreover, there is the possibility that lactate leaves brain tissue via a venous affluent and that lactate may accumulate in brain tissue downstream. In addition to lactate and glucose other energy-related substrates, such as pyruvate and in particular alanine, may also be transported via the ICS and these substrates may even leave the brain. If the latter is the case, the present turnover values lead to an underestimation of the ICS turn-over rates. Nevertheless some uncertainties, the present investigation has to be considered as a first approximation to assess the ICS turn-over rates of energy substrates in the conscious rat.

9.4.3 Implications of the extracellular turnover rates

The consumption of energy of the rat striatum is estimated to be 0.82 µmol/g/minute. The present results suggest that maximally 10% of the energy metabolites traffic through the ICS, implying that the majority of energy substrates do not traffic through this compartment. A compartment model of brain glucose suggests a reservoir behind the blood-brain-barrier and recently it has been suggested that glial glycogen is such a reservoir. One consequence of this model is that extracellular glucose is not derived directly from the bloodstream, but is delivered by astrocytes. Lactate is, besides through increased glycolysis under normoxic conditions, often thought to be produced following anaerobic glycolysis of astrocytes. These and other findings indicate that astrocyte glycolysis plays an important role in regional energy homeostasis in brain in response to increases in local neuronal activity. According to the glutamate-recycling-shuttle hypothesis of Magistretti and coworkers only lactate coupled to glutamate neurotransmission should traffic through the ICS. Indeed, in the present investigation the relative amount of glucose diffusing through the ICS is far smaller than that of lactate (<1% versus > 7% of total energy consumption). Attwell and Laughlin came to the conclusion that the glutamate-recycling shuttle may account for 3% of the total energy consumption, whereas far the most energy (80% or more) was spend in action potentials and postsynaptic effects evoked by e.g. glutamate. For general maintenance and resting potentials about 15% of the total energy expenditure was required.
Although our data do not support the suggestion that the majority of brain energy need is due to the glutamate-recycling shuttle\textsuperscript{49}, they emphasize that lactate, rather than glucose, is the major trafficking energy substrate. The present results suggest that under these conditions less than 10% of the glucose and lactate consumed by the brain is transported via the ICS. It can be argued that the present experiments are done at low activity of e.g. the glutaminergic neurotransmission, thus underestimating the contribution of ICS glucose and lactate in the activated brain. Rat striatal ICS-lactate is increased during severe physical exercise to a (maximum) increase of only 30%\textsuperscript{51}. This increase was mediated by glutaminergic innervation (presumably from the cerebral cortex as it appears to be sensitive to N-methyl-D-aspartate receptor blockade\textsuperscript{42,52}. These observations suggest that the lactate response is due to both released glutamate and glutamate receptor mediated signaling. Because the $k_2$-values were concentration-independent (see our previous discussion), the relative contribution is also not more than 30% (i.e. still less than 13% of the total energy expenditure). Moreover, during enhanced energy demand a large proportion of the glucose and lactate consumption will be utilized for (postsynaptic) signaling, rather than in the glutamate-lactate-cycle\textsuperscript{50}. So the low figure of 10% estimated with the present approach is not necessarily substantial higher during neuronal activation.

9.5 Conclusions

The present study provides estimates of extracellular turnover rates of glucose and lactate in the rat striatum. The turnover rates of lactate $0.089 \pm 0.016$ µMol/g/min and of glucose $0.024 \pm 0.003$ µMol/g/min were obtained. Together these figures show that less than 10% of rat striatal energy substrates is transferred via the ICS. This amount is compatible with the relative contribution of the glucose-lactate shuttle as estimated from a total energy balance.

9.6 References


Turnover brain extracellular glucose and lactate
