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Brain metabolism

Cerebral energy substrates
Cerebral energy consumption is very high: although the human mature brain only weighs 2-3% of the total body weight, it may consume up to 25% of the body’s energy supply (Clarke and Sokoloff, 1994; Wiesinger et al., 1997). Glucose is the primary energy substrate of the brain. In cerebral energy metabolism, one glucose molecule and six oxygen molecules ultimately lead to carbon dioxide and water. In this process, mainly in the citric acid cycle, energy is created from glucose in the form of 36 molecules of ATP (Stryer, 1995a) (figure 1-1). Energy formation can also be anaerobic, in which glucose is degraded to lactate without oxygen. This process is less efficient, as it only produces 2 ATP (Stryer, 1995b). Conventionally it is thought that lactate is formed only in emergency situations (e.g. hypoxia). The current view is that lactate is formed during most neuronal activation, without apparent oxygen deficiency.

Figure 1-1: The concept of glucose metabolism. Glucose can be derived from glycogen. Through glycolysis, glucose is transformed into pyruvate, which yields 2 ATP. Lactate is anaerobically formed from pyruvate. With oxygen, pyruvate is metabolized in the citric acid cycle, which yields another 34 ATP.

It was long thought that glucose was the only energy source of the brain, as arterio-venous uptake studies showed that only glucose and oxygen are taken up by the brain in sufficient amounts to support cerebral metabolism (Clarke and Sokoloff, 1994). Although glucose indeed is the most important energy substrate of the brain, also lactate, and ketone bodies can be used by the brain (Hasse dbalch et al., 1994; Maran et al., 1994; Hasselbalch et al., 1996; Chen et al., 2000a). However, ketone bodies are mainly used during pathological situations, when glucose levels are low (e.g. fasting, ischemia). Lactate can be used by the brain during normoglycemia and pathological situations; lactate metabolism by neurons has
been observed both in vitro (Schurr et al., 1988; Schurr et al., 1997a; Schurr et al., 1997b; Schurr et al., 1997c; Schurr et al., 1988) and in vivo (Hassel and Brathe, 2000; Qu et al., 2000; Chen et al., 2000b). Lactate thus performs a double role; it is formed when oxygen levels are low and energy is acquired anaerobically, but it can also be used as a metabolic substrate in the presence of oxygen.

Brain anatomy and blood supply

Neurons are the best known cell type in the brain, but are accompanied by a large number of other cells, gathered under the name glia (figure 1-2). Glial cells were first thought of as merely “nerve glue” (Virchow, 1871), with no other task than to keep neurons together. It is now known that each glial cell type has its own function: microglia are the macrophages of the brain, that become activated during inflammation (Peters et al., 1991), Schwann cells and oligodendrocytes sheath the axons and cell bodies in the periphery and central nervous system respectively, for support and increased conductivity (Peters et al., 1991). Finally, astroglia are star-shaped glial cells, that form an intervening layer between the neurons and the blood vessels. Therefore, capillaries and neurons are never in direct contact (figure 1-3) (Forsyth et al., 1996), as long astrocytic processes, called end-feet, ensheath both the capillary wall and the neuronal synaptic contacts (Peters et al., 1991). Because of the peculiar anatomical placement of astrocytes, which was first discovered over a hundred years ago (Golgi, 1903), it was suggested that astrocytes could play a role in cerebral nutrition (Golgi, 1903; Wolff, 1970).

As cerebral energy reserves, in the form of glycogen, are very low, cerebral nutrition is mainly supported by the cerebral blood flow (CBF, approximately 12% of cardiac output (Martini et al., 2001)). The CBF is often used as an index for neuronal activity, based on the concept of a tight coupling between neuronal functioning and the local brain energy demand (Gsell et al., 2000). The concept of coupling between changes in neuronal activity and CBF was first suggested by Roy and Sherrington (1890). Since glycogen reserves are very low, each variation of neuronal activity should lead to an increase in local brain

Figure 1-2: Three types of glial cells. Astrocytes (A) have long processes that ensheath both capillary walls and synaptic contacts. It is suggested that astrocytes could play a role in cerebral nutrition. Oligodendrocytes (B) sheath the axons and cell bodies in the central nervous system, for support and increased conductivity. Microglial cells (C) are the macrophages of the brain, which become activated during inflammation.
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metabolic demand, that can be satisfied only through an increase in the energetic supply, and thereby blood perfusion (Gsell et al., 2000). Thus, the CBF is supposed to be dynamically and precisely adjusted to metabolism, thereby reflecting underlying neuronal activity (Gsell et al., 2000).

Debate on energy metabolism

There has been a recent renewal of interest in the possible coupling of astrocytes and neurons in cerebral metabolism. It has been suggested that glucose, taken up by the brain, first enters the astrocytic end feet instead of the extracellular space. The neuron is thus, both functionally and anatomically, downstream of the astrocyte, and may receive energetic substrates from the blood via the astrocytes (Forsyth et al., 1996). Because glucose is the main energy source of the brain, the question has been whether the function of astrocytes is restricted to glucose uptake and distribution, without further metabolic processing, to neurons, or whether astrocytes release a metabolic intermediate of glucose (Tsacopoulos and Magistretti, 1996). As there are as yet no monitoring methods that can measure in vivo at cellular resolution, it is only possible to model the in vivo situation by using simpler neural systems such as the retina, or in vitro preparations of neurons and glia (Tsacopoulos and Magistretti, 1996).

With these simpler models, various evidence is found suggesting the release of lactate as an intermediate substrate by astrocytes. For example, astrocytes release large amounts of lactate (Walz and Mukerji, 1988), which is stimulated by neurotransmitters (Magistretti et al., 1986), and neurons may use lactate as an energy source (Schurr et al., 1988),(Schurr et al., 1997a; Schurr et al., 1997b; Schurr et al., 1997c). Based on this evidence, the hypothesis was raised for the astrocyte-neuron lactate shuttle (Dringen et al., 1993; Pellerin and Magistretti, 1994; Tsacopoulos and Magistretti, 1996; Pellerin et al., 1998a), in which lactate, released by astrocytes, is proposed as the metabolic neuronal substrate during
neuronal activity (figure 1-4). In response to neuronal activation at glutamatergic synapses, astrocytes increase glucose use and lactate production, despite sufficient oxygen levels to support complete aerobic metabolism (Pellerin and Magistretti, 2003). The astrocytically produced lactate is released into the extracellular space, from where it is taken up and aerobically metabolized by neurons during activity. Next to the lactate used during neuronal activity, glucose is used by neurons at rest, and presumably also during activation (Pellerin and Magistretti, 1994, 2003).

Several findings support the astrocyte-neuron lactate shuttle hypothesis. For example, there are differences in enzyme subtypes and lactate transporter affinities between astrocytes and neurons (Bittar et al., 1996; Broer et al., 1997; Pellerin et al., 1998a; Pellerin et al., 1998b; Deitmer, 2001). Neurons mainly express the enzyme lactate dehydrogenase LDH-1, which catalyzes the reaction from lactate to pyruvate, whereas astrocytes mainly express LDH-5, which catalyzes pyruvate to lactate (Bittar et al., 1996). Lactate is transported through monocarboxylate transporters (MCT), which also transport e.g. pyruvate and ketone bodies (Halestrap and Price, 1999). There are several MCT subtypes (Halestrap and Price, 1999), of which MCT1 is mostly found in astrocytes, whereas MCT2 is more neuron-specific.
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(Broer et al., 1997; Pellerin et al., 1998b). Affinity studies suggest that the glial MCT1 would favor extrusion of lactate, and the neuronal MCT2 uptake of lactate, pyruvate and other monocarboxylates (Broer et al., 1997; Broer et al., 1998; Deitmer, 2001). Both the cell type differences in LDH and MCT imply that astrocytes mainly release lactate (Deitmer, 2001), and are therefore a lactate source, whereas neurons consume lactate, and are a lactate sink (Pellerin et al., 1998a). Another finding in support of the astrocyte-neuron lactate shuttle, is that glycogen stores and accompanying enzymes, are only found in astrocytes (Peters et al., 1991; Swanson and Choi, 1993; Wiesinger et al., 1997), and that the mobilization of glycogen in astrocytes is caused by activation of neuronal circuits (Swanson and Choi, 1993; Pellerin and Magistretti, 1994; Tsacopoulos and Magistretti, 1996). This supports the idea that astrocytes are the site of intense glucose metabolism, that is at least in part induced by neurons (Tsacopoulos and Magistretti, 1996). Moreover, lactate may have some advantages over glucose as a substrate during neuronal activity, as it can be converted to pyruvate without the use of ATP, which can be very useful after hypoxia (Bliss and Sapolsky, 2001). The presence of the astrocyte-neuron lactate shuttle is suggested by the positive influence of lactate in the recovery of synaptic activity following ischemia (Schurr et al., 1988; Izumi et al., 1997; Schurr et al., 1997b; Yamagata et al., 2000) or traumatic brain injury (Chen et al., 2000b).

However, numerous findings described in literature appear not to fit perfectly into the astrocyte-neuron lactate shuttle hypothesis (Mangia et al., 2003). For example, glucose is completely oxidatively metabolized in astrocytes (Dienel and Hertz, 2001), and there are highly efficient glucose transporters in both neurons (GLUT-3) and astrocytes (GLUT-1) (Vannucci et al., 1997), suggesting glucose uptake in both cell types. Also, lactate kinetics appears to be too slow to react to fast neuronal processes; there is a slow rate of lactate uptake in astrocytes and neurons (Dienel and Hertz, 2001), and lactate increases lag behind peak neuronal activity (Chih et al., 2001). Moreover, as pyruvate and lactate levels can alter and inhibit kinetics of lactate dehydrogenase enzymes, the differential expression of LDH-1 in neurons and LDH-5 in astrocytes does not necessarily imply that astrocytes are a source of lactate and neurons act as a lactate sink (Chih et al., 2001). Also, astrocytes fail to respond to neuronal activity indicators (Takahashi et al., 1995), and during prolonged stimulation, neurons utilize neuronal pyruvate rather than astrocytic glycolysis products (Gjedde and Marrett, 2001). Finally, contrary to predictions in the astrocyte-neuron lactate shuttle hypothesis, where lactate is solely formed in the brain itself, lactate can easily pass the BBB (Oldendorf, 1971; Lear and Kasliwal, 1991; Knudsen et al., 1991). Critics of the astrocyte-neuron lactate shuttle support a more conventional view of brain metabolism, in which glucose is the principal energy source of both astrocytes and neurons. The conventional hypothesis contends that glucose is the primary substrate but does not exclude the possibility that lactate can be used as an alternative substrate under certain conditions, however, this mainly takes place in cells with low glycolytic enzymes, such as oligodendrocytes, and when glucose is temporally unavailable (Chih and Roberts, 2003).

Thus, a number of critical issues, including the possibility of preferential generation and use of lactate by astrocytes and neurons, metabolic response of astrocytes to neuronal activity, and metabolic fluxes through various transporters, are still unresolved, and current concepts of neuron-astrocyte metabolic interactions are the subject of considerable debate (Magistretti and Pellerin, 1999; Magistretti et al., 1999; Chih et al., 2001; Attwell and Laughlin, 2001; Dienel and Hertz, 2001; Gjedde and Marrett, 2001; Gjedde et al., 2002; Dienel and Cruz, 2003).
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**Monitoring brain damage**
Monitoring brain metabolism is useful not only for the basic understanding of brain functions, but also to comprehend and treat brain damage. There has been considerable interest in the development and use of biochemical measures to assess the extent of brain damage and to improve the prediction of outcome. Monitoring can be useful in any situation in which it is necessary to monitor the condition of the brain, for example, following stroke, traumatic brain injury, or intoxication with e.g. cyanide or carbon monoxide.

Ischemia/hypoxia occurs when the brain tissue is not able to extract enough oxygen and glucose from the blood due to the decreased tissue perfusion (Milde, 1989). After interruption of the blood supply (for example due to a heart attack or stroke) the limited stores of glycogen, glucose and oxygen are rapidly used up (Ljunggren et al., 1974; Wiesinger et al., 1997; Choi et al., 2003; Oz et al., 2003). During hypoxia, the brain has no other choice than to convert glucose anaerobically. A large increase in glycolytic glucose consumption and an equally large increase in lactate production are typical responses of the brain to ischemia (Schurr et al., 1999). Anaerobic glycolysis provides the adult brain with a limited amount of energy and time to maintain ion homeostasis and other essential processes before several events occur that leads to brain cell damage and death (Schurr and Rigor, 1998). During complete cerebral ischemia, the EEG becomes isoelectric within 15 to 25 seconds, the high-energy phosphate stores of phosphocreatines approach zero in four minutes, and ATP stores approach zero in 5 to 7 minutes (Milde, 1989). The energy exhaustion results in intracellular influx of calcium, release of glutamate, and mitochondrial dysfunction, eventually leading to brain edema and cell death (Milde, 1989; Castillo et al., 1996; Nagahiro et al., 1998; Dirnagl et al., 1999). Besides primary damage, secondary injury (edema, inflammation, vasospasm) contributes significantly to the extent of brain damage after head injury and stroke (Milde, 1989; Hillered and Persson, 1999; Stanimirovic and Satoh, 2000).

Specific markers for cerebral damage, which can be estimated from venous blood samples, would be a great help in establishing the extent, reversibility and recovery of ischemic tissue, provided that they can pass through the BBB and that the concentration in blood becomes sufficiently high to allow detection (Stevens et al., 1999). Metabolism markers such as glucose and lactate have concentrations that are high enough to be measured in the blood, and metabolism of both substrates is altered during brain damage (Frerichs et al., 1990; Malisz et al., 1998; Schurr et al., 1999; Glenn et al., 2003), making them excellent candidates for the monitoring of brain damage.

**Methods for monitoring brain metabolism**

*In vitro*
Metabolism can be measured in various ways. Two distinctly different measuring methods are the in vitro and in vivo measurement techniques, which can be differentiated by the lack or presence of communication and influence of other bodily systems. The in vitro method allows investigating single-type cell cultures, mixed cell cultures, acute slices, and slice cultures, with the major advantage of constant monitoring conditions and the ability to
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control the experimental conditions. A drawback of in vitro monitoring is that it is only a model of the in vivo reality. In vivo monitoring reflects the actual situation better, but can be more difficult to interpret due to a more complex system and the variable inter-subject monitoring conditions.

The brain can be represented in vitro by several set-ups, of which the acute slice preparation, organotypic slice cultures, and primary mixed or pure cultures of various cerebral cell types are the most used. Decreasing levels of interaction distinguish these various in vitro set-ups. Organotypic slice cultures are cultured slices of the neonatal brain, mostly the hippocampus, and offer conditions where astrocyte neuronal coupling, and the intrinsic neuronal circuits are minimally disturbed during the explantation procedure, unlike, for instance, re-aggregated mixed neuronal/glial cultures (Gramsbergen et al., 2003). In both mixed cultures and hippocampal slices, it is not possible to distinguish between the various cell types. The metabolism of a particular cell type can be studied in pure cell cultures, thus without interaction with other cell types. This is therefore the simplest representation of brain metabolism.

Cell cultures are often used to study cellular and brain metabolism. In fact, a significant part of the astrocyte-neuron lactate shuttle hypothesis (for example, a large release of lactate by astrocytes (Walz and Mukerji, 1988), and the mandatory role of lactate in the recovery of synaptic activity following ischemia (Schurr et al., 1988; Izumi et al., 1997; Schurr et al., 1997b; Yamagata et al., 2000)) is based on in vitro evidence, again, mainly because of the difficulty to measure cellular energy metabolism in vivo.

In vivo

In the body, monitoring metabolism of the brain is challenging. The brain is situated inside the skull, making the brain not easy to approach. Various solutions have been invented to nevertheless measure brain metabolism in vivo, for example: imaging (e.g. PET, MRI), intracerebral microdialysis, and measurements of arteriovenous differences.

Imaging is non-invasive, but can only be performed occasionally in dedicated locations, and is thus not suitable for frequent monitoring. Intracerebral microdialysis is more suitable for the frequent analysis of metabolic substrates, but has as disadvantage that the dialysis probe can only measure in its direct environment, and thus does not inform about the condition of the total brain. Moreover, the placement of a microdialysis probe is very invasive and relevant data can only be obtained for a limited time period (Leegsma-Vogt et al., 2001).

Overall metabolism of the brain can also be studied by the measurement of arterio-venous differences. In this method the uptake or release of substances by the brain can be calculated from the concentration-difference in the arterial and venous blood. One of the first studies to use arterio-venous measurements was the measurement of cerebral blood flow and metabolism in man using nitrous oxide (Kety and Schmidt, 1945), later known as the Kety-Schmidt technique. The technique enables simultaneous determination of cerebral metabolic rates for oxygen, glucose and lactate (Linde et al., 1999), and is considered the gold standard for quantitative determination of global levels of cerebral blood flow (Lassen, 1985). The Kety-Schmidt technique has much been applied using various other tracers (for example, xenon, (Linde et al., 1999)).

Using other tracers, it is also possible to measure uptake and metabolism by the measurement of these tracers in the brain itself. One of the most used and best known
tracers is deoxyglucose (DG). Firstly developed by Sokoloff et al (1977), DG was used to measure glucose consumption rates in the various structural and functional components of the brain in vivo. DG (in the forms of [14C]DG, 2-DG, or fluoro-DG) is used because the label in its end product, deoxyglucose-6-phosphate, is trapped in the tissue over the time course of the measurement, and can there be quantified (Sokoloff et al., 1977), assuming that the uptake of DG reflects glucose metabolism. For the DG method, arterio-venous measurements become less important, and only arterial levels are measured (Sokoloff, 1981). Nowadays, 2-deoxyglucose (2DG) or fluoro-deoxyglucose (FDG) is mostly used in combination with imaging techniques (Alavi et al., 1986; Magistretti and Pellerin, 1996; Magistretti and Pellerin, 1999).

The main arterio-venous measurement techniques used today are the Kety-Schmidt technique, and the “plain” measurement of AV-differences across the brain. The arterio-venous difference method has among others been used to study glucose, lactate and oxygen levels during and after (sub-) maximal exercise in humans (Ide et al., 1999; Ide et al., 2000), lactate differences in patients with severe head injury (Murr et al., 1996), and the glucose, oxygen and lactate consumption before, during and after generalized stimulation in rats (Madsen et al., 1999). The use of arterial and venous catheters in patients is an already normal clinical practice, and far less invasive as the placement of a probe in the brain (Leegsma-Vogt et al., 2001).

**Batch-wise versus on-line monitoring**

Samples can be measured batch-wise or on-line. The batch-wise measurement is mostly used, in which the effect of a stimulus is measured in representative samples. Samples are often not measured directly, but preserved for transfer to and measurement in the laboratory (for example, by autoradiography or immunocytochemistry). In the batch-wise approach there are relatively few measurement points.

The on-line method however, has, as the name suggests, a high temporal resolution due to the (semi-)continuous measurement. The monitoring equipment is directly connected with the sample, creating opportunities for instant measurement and many sample points. This could be used, for example, for immediate therapeutic intervention in patients due to bedside analysis of the patient’s condition. The on-line measurement is more difficult to perform than the batch-wise approach, as sophisticated equipment is needed to monitor and analyze metabolism directly. On-line measurement is a very young technique, and is as yet not much applied. The few studies that use on-line monitoring include the intravenous and subcutaneous measurement of glucose using on-line microdialysis (Fang et al., 1997), the on-line monitoring of radiolabeled fluoro-deoxyglucose uptake in human glioma cells (Noll et al., 2000), and the on-line monitoring of biomass in hybridoma cells (Noll and Biselli, 1998).

**Our on-line system**

The on-line system used in this thesis was originally developed by Elekes et al. (1995). Basically, it consists of a flow injection analysis (FIA) system with biosensors for glucose and lactate. The FIA system allows (semi-) continuous measurement due to sample injection: a sample is injected at a preset time interval into a constant flow, which passes the measuring devices (in our case: biosensors, see below) and electrodes where electron transfer is measured. The major advantage of FIA is the recurrent return to baseline voltage,
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thereby enabling to monitor the stability of the system. Using calibration curves, voltages are converted to concentrations. The sample injection can be set at various time intervals; in most studies we have measured samples every minute. By using very small tubing (50-100 µm internal diameter, 20-80 nL/cm) throughout the system, both the lag-time and blending of the samples is minimized (Kaptein et al., 1997; Savenije B et al., 2002).

The FIA system can be easily modified. The simplest FIA set-up (figure 1-5) includes the sample to be measured, the FIA system itself, two systems that generate flow through the system, and (bio)sensors and electrochemical flowcells for actual measurement. This system can be expanded to serve the experimenter’s needs: the FIA analysis system has for example been used to measure subcutaneous glucose levels (Kaptein et al., 1998; Tiessen et al., 2002) and intravenous glucose and lactate concentrations after myocardial infarction (Tiessen et al., 2001), and arterio-venous glucose and lactate differences (this thesis). In vivo samples have to be pre-filtered as subcutaneous fluid and blood contain chemicals and cells that contaminate the measuring system. In our in vivo experiments, we used ultrafiltration as a selection/filtration technique.

Figure 1-5: An example of a flow injection analysis (FIA) set-up, used for in vitro monitoring. Arrows indicate flow direction. Medium is pulled from the oxygenized medium through the cell chamber by a syringe pump, which can be adjusted to various flow rates. Every minute a sample from the cell chamber is transferred to the glucose and lactate biosensors by the sample injector, and currents are detected by electrochemical detectors and recorded. The FIA system can be adjusted (e.g. doubled, various biosensors) to serve the experimenter’s needs.

Biosensors

Biosensors are electrodes or other surfaces covered with a protein that can bind or convert a substrate, thereby creating an electrical signal (Kaptein, 1998; Korf et al., 2003). The selectivity of a biosensor is dependent on the properties of the protein: mostly an enzyme, sometimes an antibody or receptor. The signal is ideally specific, and representative of the concentration of the substrate detected (Pickup, 1985; De Boer, 1993; Kaptein, 1998; Tiessen, 2001; Korf et al., 2003). Various authors have described the production of biosensors, and a variety of immobilized enzymes is utilized in order to obtain selectivity of the biosensor and to overcome interferences from electroactive species (Rhemrev-Boom,
Biosensor applications for off-line discontinuous monitoring have well been documented, but for continuous in vivo monitoring little data have been reported (Rhemrev-Boom, 1999). There are several types of biosensors, for example, transcutaneously placed, microdialysis- or ultrafiltration-based, needle-type, and sandwich-type biosensors (Korf et al., 2003).

In this thesis we have worked with a sandwich-type biosensor (figure 1-6). This biosensor measures glucose and lactate by using glucose oxidase or lactate oxidase respectively. In the sandwich-type sensor, originally developed by Flentge et al. (1992), the glucose or lactate oxidase enzyme is combined with horseradish peroxidase, which are jointly physically immobilized between two cellulose nitrate filters, which are located between two stainless steel screens that prevent the obstruction of flow through the biosensor.

In conventional electrochemical methods, the consumption of oxygen or the production of H₂O₂ is monitored. However, these are often affected by the oxygen concentration, and the oxidation of hydrogen peroxide requires a large overpotential that may also detect electro-oxidable contaminants (Elekes et al., 1995). In the sandwich-type biosensor, these problems are avoided by using a combination of horseradish peroxidase and a ferrocene derivative (Fc) as a mediator, in which the ferrocene reduction current related to the concentration of hydrogen peroxide is monitored amperometrically (frew J.E. et al., 1986; Elekes et al., 1995).
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1995). By using the second enzymatic reaction and the mediator, two advantages are obtained. First, it allows low potential detection of substrates (0 mV vs. Ag/AgCl) thus avoiding interferences of electroactive species and ensuring the activity of the assay. Second, the assay sensitivity is enhanced as a result of increasing efficiency of the electrochemical detection, as decay of the formed H₂O₂ is very low (Elekes et al., 1995). A major advantage of the use of ferrocene is that the interference by ascorbic acid is diminished, so that the assay becomes highly specific (Elekes et al., 1995). The enzyme immobilization method is simple, thus avoiding the possible enzyme denaturation that occurs often in more complicated immobilization procedures, and the amount of the immobilized enzyme is not dependent of the accessibility of binding sites, as is often limiting with chemical immobilization (Elekes et al., 1995). Therefore, this biosensor is cost effective since a small amount of enzymes can be used for a large number of determinations possessing an excellent operating life (Elekes et al., 1995). Both the lactate and glucose reactor remains active for at least 6 weeks and 10,000 or more determinations. This method is applicable for other oxidizing enzymes as well, leading to several analytical possibilities.

OUTLINE OF THIS THESIS

Neither metabolism of the brain in vivo, nor energy use of brain cells in vitro, has been monitored with our on-line system before. In this thesis we have measured glucose and lactate metabolism on-line, both in vitro and in vivo. The on-line system with enzyme-based biosensors allows to measure glucose and lactate concentrations very specifically, whereas the small tubing and very low volumes give us high time resolution and low lag times. We investigated the use of the on-line monitoring system for in vitro and in vivo measurement of brain metabolism, and studied today’s concepts on cellular and cerebral metabolism.

This thesis is divided into two parts. The first part focuses on the vitro measurement of cellular glucose and lactate metabolism, the second part concentrates on the in vivo measurements of the cerebral glucose and lactate use.

Part 1 begins with the description of the novel in vitro monitoring technique for cell cultures (chapter 2). We designed a cell chamber, allowing continuous perfusion of cell cultures. We measured glucose and lactate metabolism at a minute-to-minute time resolution. The use of our perfusion-detection technique for in vitro monitoring is demonstrated in a wide variety of cells, including primary neuronal and astroglial cultures, yeast cells and human lymphocytes. The potential of in vitro on-line monitoring is discussed for application in studying normal and abnormal metabolism, toxic and non-toxic drug effects, and human tissue biopsies.

Chapter 3 presents the measurement of glucose and lactate metabolism in organotypic hippocampal slice cultures. Contrary to cell cultures, organotypic hippocampal slices are an in vitro continuation of an in vivo created cellular complex. Both neurons and astrocytes are present in the slice cultures, and the net metabolism of this cellular complex was studied with the same experimental set-up, but with a slightly different cell chamber than described in chapter 2. Quantitative glucose and lactate metabolism was assessed in continuously perfused organotypic hippocampal slices, under control conditions and during exposure to glutamate and drugs that interfere with aerobic and anaerobic metabolism. We found that
50% of consumed glucose was converted to lactate, and lactate efflux from glycogen stores is suggested.

Complementary to studying the net metabolism of a combined neuronal-astrocytic culture, we investigated the energy use of an astrocytic monoculture in chapter 4. Astrocytes are known to release lactate, and are suggested to have a feeding role for neurons. In this chapter we investigated glucose and lactate metabolism in primary astrocytic cultures, using the in vitro on-line monitoring unit. Astrocytes were offered glucose, lactate or a combination of both substrates. In this study we found indications for glycogen breakdown in cell culture metabolism, and the results suggest a separate lactate pool in cell culture metabolism.

In part 2, the in vivo measurement of glucose and lactate metabolism is addressed. We begin with a review on the use of biosensors for in vivo monitoring (chapter 5), in which both subcutaneous as intravenous studies are described. We explain the principle of biosensors, and describe their application in clinical monitoring and experimental research. Chapter 6 continues with a review on the ultrafiltration (UF) technique, which is a convenient technique for frequent in vivo sampling. The review explains the principles of UF, and describes the similarities and differences between microdialysis (MD) and UF, discussing the advantages and disadvantages of UF as compared to MD. The potential of in vivo UF as a continuous tissue sampling technique is discussed.

The in vivo empirical studies begin in chapter 7, where ultrafiltration and the in vivo online sampling system is used to monitor brain metabolism during and after brain damage in rats. The net uptake and release of glucose and lactate was calculated using arterio-venous glucose and lactate measurements. Continuous monitoring of arterio-venous glucose and lactate differences may serve as a diagnostic tool to assess normal brain function and brain pathology. The finding that the flux of lactate in and out of the brain is not only dependent on the lactate concentration in the brain, but on blood levels as well, was used in the following study (chapter 8), in which evidence was found for a lactate pool in the rat brain that is not used as an energy supply. We studied lactate metabolism by the brain in rats by increasing the lactate concentration in the blood. The results suggest that the majority of lactate moving into the brain does not serve as an energy substrate, and that lactate does not replace glucose as an energy source. From the lactate infusion data, we mathematically modeled lactate metabolism in vivo in chapter 9. Our experiments allow describing lactate kinetics in individual experiments with a high temporal resolution. From both the mathematical model and the measured data we found evidence that the lactate distribution volume increases with infusion length; we theorize that the cerebral lactate distribution volume is approximately 24% of the brain volume. Finally, in chapter 10, the findings from chapters 2-9 are summarized and discussed.

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GENERAL INTRODUCTION


