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Summary and Discussion
CHAPTER 10

SUMMARY

In this thesis we have measured brain glucose and lactate metabolism, using a novel on-line monitoring method. Challenges were found in the application of the on-line system for monitoring in vivo and in vitro glucose and lactate metabolism, and in the interpretation of data into the current debate on brain substrate use.

The utilization of the monitoring system for in vitro measurements, and the use of cell cultures to measure brain metabolism was evaluated in Chapter 2, where the technique of quantitative in vitro monitoring was explained. Although several other flow-through systems have been reported (Gramsbergen et al., 2003; Cucullo et al., 2002; Gloeckner and Lemke, 2001; Noll et al., 2000; Noll and Biselli, 1998), on-line monitoring systems have never been described for cell preparations. To monitor cellular metabolism on-line, a special cell chamber was developed. The chamber was specially designed to handle low flow rates, essential to achieve minimal dilution of the analytes. Glucose consumption and lactate efflux were measured in various cell types to inform about energy use and adequate oxygen delivery. In all of our experiments, the calculated aerobic ratio was flow rate dependent, and reached up to 90%. The calculated aerobic ratio is in the upper range of estimated aerobic ratios in cell cultures (10-75%, calculated from Cucullo et al., 2002; Gloeckner et al., 2001). The results implied that the calculated aerobic ratio could be an underestimation of the real value, as there was evidence that lactate may be (partly) derived from glycogen instead of glucose. An innovative advantage of the on-line monitoring system is that metabolism can be calculated per cell. The (preliminary) data in this chapter suggest that neurons and astrocytes metabolize glucose in the same order of magnitude, whereas yeast cells consume a hundred times less glucose per cell.

Chapter 3 continues with the description of an in vitro monitoring system for organotypic hippocampal slice cultures. Glucose metabolism and lactate production have not been studied before in cultured brain slices. A characteristic of organotypic hippocampal slice cultures is, that the astrocyte-neuronal coupling is minimally disturbed during the explantation procedure, and that, in contrast to acute slices, the tissue is in a stable condition at the start of the experiment. In this chapter, a different tissue chamber type was used in combination with our flow-injection on-line monitoring system. Quantitative glucose and lactate metabolism was assessed in continuously perfused hippocampal slices, under control conditions and during exposure to glutamate and drugs that interfere with aerobic and anaerobic metabolism. In these slice culture experiments, about 50% of consumed glucose was converted to lactate, and there was significant lactate uptake, although this uptake was lower than the uptake of glucose. Moreover, glucose deprivation experiments suggested lactate efflux from glycogen stores. The study in Chapter 3 demonstrated that there is both lactate release and lactate re-uptake under basal conditions, that lactate uptake is less efficient than glucose uptake, and that maintaining Na+ and K+ gradients consumes much of the energy and is linked to glycolysis.

Complementary to monitoring metabolism in hippocampal slices, we studied glucose and lactate metabolism in primary astrocytic cultures in Chapter 4. Astrocytes are strategically positioned between the capillaries and the neurons (Forsyth et al., 1996), and are suggested to play a feeding role for neurons (Pellerin and Magistretti, 2003; Magistretti et al., 1999; Pellerin et al., 1998). Glucose and lactate uptake/release was measured while the cells were exposed to glucose, lactate or a combination of both substrates. In this chapter we expanded the preliminary results on substrate use per cell, and estimated glucose and lactate
consumption per cell per minute. We found that glucose was the most preferred substrate; glucose use was approximately 3.5 femtomoles/cell/minute. There were indications for glycogen breakdown from large glycogen stores in astrocytes, which mainly resulted in lactate release. Glucose use did not change when lactate was provided in the medium, which led to the suggestion that there might be a separate pool for lactate, as glucose use would become lower if lactate were used as a replacement for glucose. Calculations on glycogen content and energy use per cell indicated that the energy use and supply per cell in vivo is much lower than energy use in vitro. Such observation can have significant implications in the consideration of significance of in vitro results to understand in vivo observations and hypotheses.

Part 2 of this thesis focused on the in vivo monitoring of brain metabolism, and started with a review on the use of biosensors for in vivo monitoring (Chapter 5). In this chapter we explained the working of these biosensors, and described their application in clinical monitoring and experimental research. The mini-review illustrated that biosensors can be applied in different areas of experimental and clinical research; e.g. brain trauma, myocardial infarction and diabetic care. The biosensors created in our laboratory are cost effective, and have an excellent operating life: both the lactate and glucose reactor remains active for at least 6 weeks and 10,000 or more determinations. The review aimed to demonstrate that biosensors, combined with ultrafiltration or microdialysis devices, are useful in monitoring glucose levels and pathological events, both subcutaneously and intravenously.

The thesis continued with a mini-review on ultrafiltration (UF) as an interface method for in vivo sampling (Chapter 6). The review discussed the potential of in vivo UF as a continuous tissue sampling technique in clinical research areas, and in several major biomedical applications including glucose and lactate monitoring and drug kinetic studies. The subcutaneously or intravenously placed UF probes have been studied for off-line sample analysis and for continuous on-line monitoring, in a wide variety of species, including dogs, rats, pigs and humans. UF is much alike microdialysis (MD), but there are important differences, which were addressed in this chapter. This review aimed to point out that UF can be a good alternative for MD.

The experimental section of part 2 started with a study on in vivo glucose and lactate monitoring after brain damage in rats (Chapter 7). New in this chapter was the application of two ultrafiltration probes in a single animal to monitor glucose and lactate concentrations at two locations simultaneously. Both the method and results obtained with arterio-venous measurements in the halothane anaesthetized rat before and after brain injury were described. Net cerebral lactate efflux and glucose uptake was seen under control conditions and at low blood lactate levels. After injuring the brain by balloon inflation, there was a decrease in the glucose utilization and a slightly lower efflux of lactate, demonstrating the potential of arterio-venous monitoring to assess brain damage. The results indicated 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. Apparently, the brain is not a closed system for lactate.

In Chapter 8, we further explored the concentration dependency of the lactate flux by studying lactate uptake and release by the rat brain during and after lactate infusion. Moreover, we investigated if lactate, taken directly taken from the blood circulation, could replace glucose as a cerebral energy source. The change in brain lactate and glucose flux during and after lactate infusions in the rat was investigated, by monitoring venous-arterial
(VA) differences over the brain. The flux of lactate was concentration dependent, with an influx of lactate during lactate infusion, and a net efflux of lactate immediately after the infusion. The results suggested that the majority of lactate moving into the brain was not used as an energy substrate, and that lactate does not replace glucose as an energy source. Instead, the concept of a lactate pool in the brain was proposed, that can be filled and emptied in accordance with the blood lactate concentration, but which is not used as an energy supply for cerebral metabolism.

Finally, in chapter 9, we used the lactate infusion data to investigate lactate kinetics, by theoretically modeling lactate metabolism in the body. Both the arterial and venous data were used to mathematically theorize on bodily lactate systems. Due to the high temporal resolution, arterio-venous lactate kinetics could be modeled in individual experiments. We described the delayed lactate release from the head, and found that the distribution volume in the head increased with infusion time. Calculations suggest that lactate can be dispersed in approximately 24% of brain volume.

GENERAL DISCUSSION

Methodology

In this thesis, the capability of quantitative on-line monitoring of cellular and cerebral glucose and lactate metabolism was demonstrated. The on-line monitoring system used in this thesis is characterized by small-volume tubing and low dead volumes of the connections, resulting in low lag-times (approximately 5 minutes) and high temporal resolution. By miniaturizing the system, including the biosensors (Rhemrev-Boom et al., 2002, 2003), time resolution and lag-time can be even more improved.

The potential of the utilized biosensors (chapter 5) and the FIA system is demonstrated in a wide variety of experiments, both in this thesis (chapters 2, 3, 4, 7, 8, 9) and in previous research (Tiessen et al., 2001, 2002; Kaptein et al., 1997, 1998). The system can be easily modified: two distinct set-ups of the FIA were used in this thesis (chapters 2-4 versus 7-9), but essentially, all elements can be altered according to the experimenter’s needs. For example, the biosensors can be customized very easily by simply changing the enzymes used for detection, enabling the detection of other metabolites (e.g. glutamate, uric acid, ethanol, see chapter 5).

The newly developed concept of cell/slice chambers enabled the successful on-line monitoring of a wide variety of cell types (chapters 2, 4), and organotypic hippocampal slices (chapter 3). The cells inside the cell chambers can have a flow-dependent (closed chamber, chapters 2 and 4) and flow independent (open chamber, chapter 3) aerobic metabolism, caused by the different approach of oxygen delivery to the cells. The relatively high aerobic ratio measured with both types of cell chambers suggests a healthy cellular condition, which is essential for accurate monitoring of energy metabolism. The potential of the cell/slice chamber was established in this thesis, and as in vitro on-line monitoring is a relevant and safe way to study effects of drugs and other stimuli on cellular condition, the prospect for future on-line in vitro research is favorable. For example, metabolism can be studied in isolated cell types which normally function cooperatively (e.g. neurons and glia),
ischemic resilience can be monitored in cell types, for the development of drugs for new therapeutic interventions (e.g. heart cells), and (human) biopsy samples can be studied, including chemotherapeutic agents on cancer cells. Moreover, the application of in vitro monitoring may ultimately decrease the use of laboratory animals and optimize patient care. Furthermore, on-line cellular metabolism studies with a high time resolution allows the innovative calculation of energy use/cell/minute (chapters 2 and 4). The high temporal resolution per experiment is also an advantage in the analysis of lactate kinetics per individual experiment (chapter 9), which can be exemplary for additional uses of on-line high-resolution monitoring.

Another new concept in this thesis, besides the ability to monitor in vitro metabolism, is the introduction of two monitoring units to enable arterio-venous measurements (chapters 7-9). The arterio-venous monitoring technique proved successful in measuring the uptake and release of glucose and lactate by the brain after brain trauma (chapter 7) and lactate infusion (chapters 8 and 9), although we also monitored extracerebral tissue during lactate infusion experiments (chapter 9). To monitor brain lactate kinetics, the infusion time should be as low as possible, and venous efflux should probably be measured in a different vein, nearer to the brain (e.g. the sinus). The arterio-venous monitoring technique could, of course, be applied to study the uptake and release of metabolites in various organs in the body. For the in vivo experiments, ultrafiltration was introduced as a pre-filtration method (chapter 6) as both the biosensors as the whole monitoring unit cannot process large proteins and cells. In this thesis the applicability of ultrafiltration was demonstrated, making it a useful technique in a fast array of in vivo experiments instead of, or complimentary to, microdialysis.

Metabolism

In this thesis, we have studied glucose and lactate metabolism in vitro and in vivo, and found, among other things, that glucose is the most preferred substrate (chapters 2, 3, 4, 7, 8). Half of the glucose taken up is released as lactate, both in vitro (chapter 3) as in vivo (chapter 7), indicating that lactate can easily cross the blood-brain barrier (further explored in chapter 8). Although the formation of lactate from glucose falls in line with the astrocyte-neuron lactate shuttle hypothesis, the flux of lactate across the blood-brain barrier does not, as the formation and metabolism of lactate is hypothesized to only occur in the brain itself (Pellerin et al., 1998; Tsacopoulos and Magistretti, 1996; Pellerin and Magistretti, 1994; Dringen et al., 1993). Perhaps more important, measurements on glucose uptake without consideration of lactate formation seriously overestimates glucose metabolism. This has to be taken into account for example, in deoxyglucose (DG) measurements. The DG hypothesis is based on the fact that deoxyglucose cannot be metabolized, and is therefore trapped inside the cell, indicating where glucose uptake has taken place (Sokoloff, 1981; Sokoloff et al., 1977). In literature, DG uptake has been equated with glucose metabolism (for example: Henry et al., 2001; Magistretti and Pellerin, 1996, 1999). As lactate passing over the blood-brain barrier was thought to be minimal (Cremer et al., 1979; Nemoto and Severinghaus, 1974), no calculations were incorporated on lactate efflux from brain. However, in this thesis we found a large lactate flux across the blood-brain barrier (chapter 8), indicating that the uptake of glucose, as seen with DG, highly overestimates aerobic glucose metabolism.

In our in vitro studies, we have found evidence for large glycogen stores, which vary with incubation conditions, and are broken down and released into the medium as lactate
(chapter 4). Although the lactate release from glycojen stores is in line with the astrocyte-neuron lactate shuttle hypothesis, the lack of effect of glutamate on glycolysis (chapter 3) is contradictory to the hypothesis. Moreover, the influence of incubation conditions on glycojen levels and thereby lactate output (chapter 4), and the higher energy use in vitro compared to in vivo (chapter 4), suggests that extrapolation of conclusions from in vitro studies on energy metabolism in vivo has to be considered critically. This may have profound influences on the foundations of the astrocyte-neuron lactate shuttle hypothesis, as many of the evidence supporting the hypothesis are found during in vitro investigations.

In this thesis, lactate consumption was found in vitro in neurons, astrocytes and hippocampal slices (chapters 2-4), and 4CIN inhibited the neuronal MCT transporter (chapter 3), which imports lactate into neurons. Although the neuronal lactate metabolism and the effect of 4CIN on neuronal metabolism supports the astrocyte-neuron lactate shuttle hypothesis, the finding that astrocytes consume lactate challenges the hypothesis. Contrary to the lactate use in cell cultures, no net lactate metabolism was measured by the brain in vivo (chapter 8). The lack of net lactate metabolism does not necessarily imply that lactate is not metabolized at all. In fact, studies have found lactate metabolism in the brain in vivo (Hassel and Brathe, 2000;Qu et al., 2000;Bouzier et al., 2000). Also, the decreased release of lactate after brain trauma (chapter 7) could be due to the (increased) metabolism of lactate or the metabolism of glycojen (which could be a source of lactate efflux), as metabolism of lactate after brain trauma has been suggested (Chen et al., 2000). However, it appears that this lactate metabolism does not play any role in net cerebral energy use (chapter 8). Apparently, the flux of lactate into a cerebral pool (chapter 8), comprising approximately 24% of brain volume (chapter 9), is (partly) independent of the glucose pool (as suggested by both in vitro and in vivo studies), and is based on a concentration difference across the blood-brain barrier (chapters 7 and 8), and not on the active uptake of lactate for use as a metabolite.

In summary, in this thesis we have found various evidence in favour or against the astrocyte-neuron lactate shuttle hypothesis. Although the lactate release from glucose and glycojen in astrocytes (chapters 2 and 4), lactate metabolism in neurons (chapter 2) and the effects of 4CIN on neuronal metabolism (chapter 3) support the astrocyte-neuron lactate shuttle hypothesis, the preference of astrocytes and neurons for glucose (chapters 2 and 4), the lack of effect of glutamate on metabolism (chapter 3), and lactate metabolism in astrocytes (chapter 4), speak against the hypothesis. Keeping in mind the dissimilarity between the in vitro and in vivo energy use and glycojen content, one must consider the in vitro results in favor or against the astrocyte-neuron lactate shuttle hypothesis with great caution. For example, it could be likely that the high glycojen content and the lactate release from glycojen in vitro are an artifact, caused by the incubation conditions (chapter 4). As the astrocyte-neuron lactate shuttle hypothesis is mainly based upon in vitro results, this thesis challenges the basis of that hypothesis. Although many results, from this thesis and from literature, can be explained in favor or against the astrocyte-neuron lactate shuttle hypothesis, many of those results are extrapolated from in vitro studies, and are therefore circumstantial. The best way to minimize misinterpretation, is to interpret data that are obtained with a model close to the in vivo “reality”. Unfortunately, it is extremely difficult to interpret whole-brain in vivo data, as obtained in this thesis, to cellular theories. For example, the lack of net lactate metabolism by the brain does not necessarily imply that lactate is not metabolized at all, as mentioned above. Therefore, the in vitro results are used to theorize on cellular and cerebral energy metabolism.
SUMMARY AND DISCUSSION

The major difference between the astrocyte-neuron lactate shuttle hypothesis, and the conventional hypothesis (for the description of both hypotheses, see the General Introduction, Brain metabolism: Debate on energy metabolism), is the preferential use of lactate over glucose (Chih and Roberts, 2003; Pellerin and Magistretti, 2003). Whereas the astrocyte-neuron lactate shuttle hypothesis suggests that lactate is preferred by neurons over glucose during neuronal activity, the conventional hypothesis states that lactate can be an alternative substrate to (various) brain cells, but is mainly used when glucose is temporarily unavailable (Chih and Roberts, 2003; Pellerin and Magistretti, 2003). As glucose was the preferred substrate in both neuronal and astrocytic cultures (chapters 2 and 4), and lactate was metabolized by both cell types (in a lesser extent than glucose, chapters 2 and 4), our data suggest that lactate is not preferred over glucose by neurons, and thus point towards the conventional hypothesis. Moreover, the lack of effect of glutamate on hippocampal slice metabolism (chapter 3) opposes the essence of the astrocyte-neuron lactate shuttle hypothesis. Although our in vitro data support the conventional hypothesis on brain metabolism, it may not be forgotten that the high energy use and glycogen levels in cell cultures may create artifacts and misinterpretations. Direct evidence on brain metabolism can only be found when instrumentation is created that can measure cerebral metabolism on a cellular scale. Until that time, the debate on cerebral substrate use will continue.

REFERENCE LIST


