During the last decade, important biochemical mechanisms involved in the development of irreversible ischemic brain injury have been described, for example, the effects of failing energy metabolism, excessive lactic acidosis, free radical reactions, and the neurotoxic effects of excitatory amino acids. Such mechanisms have been studied mainly in experimental models of cerebral ischemia. Apart from magnetic resonance (MR) imaging and positron emission tomography (PET) studies of brain energy metabolism and studies of arteriovenous differences in oxygen and lactate, relatively few investigations have been conducted in patients, mainly because of the lack of suitable methods. Intracerebral microdialysis is a new technique by which these mechanisms can be studied in humans. Important chemical substances involved in the development of cerebral ischemia can be retrieved from the extracellular fluid (ECF) of the brain, for example, energy-related substances such as lactate, pyruvate, glucose, and hypoxanthine, and excitatory amino acids such as glutamate and aspartate. Fluctuations in the ECF levels of these substances reflect intracellular metabolic disturbances produced by ischemia. We have recently applied and evaluated microdialysis for clinical use in brain injury research. Our basic idea was that monitoring substances in the ECF with microdialysis could provide useful information on metabolic disturbances with a high temporal and spatial resolution. We have concluded so far that microdialysis may be a promising tool for chemical monitoring of the human brain and that extracellular fluid levels of lactate, lactate/pyruvate ratio, glucose, hypoxanthine, and glutamate are useful markers of disturbances in brain energy metabolism in neurointensive care patients. These results have generated a working hypothesis that the pattern of these extracellular markers may help differentiate between various causes of energy perturbations, such as hypoxia and different degrees of ischemia. The correlation between the dialysate levels of excitatory amino acids and outcome supports the concept of glutamate receptor overactivation in acute human brain injury.

The authors have developed a method for routine monitoring of disturbances in brain energy metabolism and extracellular levels of excitatory amino acids using intracerebral microdialysis in 10 patients with subarachnoid hemorrhage. Microdialysis was conducted for periods ranging from 6 to 11 days after ictus. Altogether, 16,054 chemical analyses from 1647 dialysate samples were performed. Concentrations of the energy-related substances lactate, pyruvate, glucose, and hypoxanthine were measured, and the lactate/pyruvate ratio was calculated. The excitatory amino acids glutamate and aspartate were measured. The microdialysis data were matched with computerized tomography findings, clinical course, and outcome. The results support the concepts that microdialysis is a promising tool for chemical monitoring of the human brain and that extracellular fluid levels of lactate, lactate/pyruvate ratio, glucose, hypoxanthine, and glutamate are useful markers of disturbances in brain energy metabolism in neurointensive care patients. These results have generated a working hypothesis that the pattern of these extracellular markers may help differentiate between various causes of energy perturbations, such as hypoxia and different degrees of ischemia. The correlation between the dialysate levels of excitatory amino acids and outcome supports the concept of glutamate receptor overactivation in acute human brain injury.

Key Words • subarachnoid hemorrhage • microdialysis • cerebral ischemia • neurointensive care • lactate • glutamate
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TABLE 1

Basal cortical dialysate levels (µmol/L) of lactate, glucose, hypoxanthine, glutamate, aspartate, and lactate/pyruvate (L/P) ratio in studies using similar microdialysis systems

<table>
<thead>
<tr>
<th>Authors &amp; Year</th>
<th>Substance</th>
<th>Species</th>
<th>Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hillered, et al., 1989</td>
<td>lactate</td>
<td>rat</td>
<td>80–140</td>
</tr>
<tr>
<td>Nilsson, et al., 1990</td>
<td>lactate</td>
<td>rat</td>
<td>100–150</td>
</tr>
<tr>
<td>Hillered, et al., 1990</td>
<td>lactate</td>
<td>man</td>
<td>200–400</td>
</tr>
<tr>
<td>Fellows, et al., 1992</td>
<td>glucose</td>
<td>rat</td>
<td>100–250</td>
</tr>
<tr>
<td>Valtysson, et al., 1995</td>
<td>glucose</td>
<td>rat</td>
<td>110–240</td>
</tr>
<tr>
<td>Ronne-Engström, et al., 1995</td>
<td>glucose</td>
<td>rat</td>
<td>180–220</td>
</tr>
<tr>
<td>Nilsson, et al., 1990</td>
<td>hypoxanthine</td>
<td>rat</td>
<td>1–2</td>
</tr>
<tr>
<td>Hillered, et al., 1990</td>
<td>hypoxanthine</td>
<td>man</td>
<td>2–6</td>
</tr>
<tr>
<td>Nilsson, et al., 1990</td>
<td>glutamate</td>
<td>rat</td>
<td>1–2</td>
</tr>
<tr>
<td>Hillered, et al., 1990</td>
<td>glutamate</td>
<td>man</td>
<td>1–2</td>
</tr>
<tr>
<td>Nilsson, et al., 1994</td>
<td>glutamate</td>
<td>rat</td>
<td>0.7–1.6</td>
</tr>
<tr>
<td>Nilsson, et al., 1990</td>
<td>aspartate</td>
<td>rat</td>
<td>0.2–0.4</td>
</tr>
<tr>
<td>Hillered, et al., 1990</td>
<td>aspartate</td>
<td>man</td>
<td>0.2–0.6</td>
</tr>
<tr>
<td>Nilsson, et al., 1994</td>
<td>aspartate</td>
<td>rat</td>
<td>0.1–0.2</td>
</tr>
<tr>
<td>Hillered, et al., 1989</td>
<td>L/P ratio</td>
<td>rat</td>
<td>15</td>
</tr>
<tr>
<td>Nilsson, et al., 1990</td>
<td>L/P ratio</td>
<td>rat</td>
<td>15–20</td>
</tr>
<tr>
<td>Hillered, et al., 1990</td>
<td>L/P ratio</td>
<td>man</td>
<td>15</td>
</tr>
<tr>
<td>Persson &amp; Hillered, 1992</td>
<td>L/P ratio</td>
<td>various</td>
<td>11–20</td>
</tr>
</tbody>
</table>

* Values are the means or the means ± standard deviation as indicated in the cited references.

Microdialysis data and other signs of cerebral ischemia, particularly computerized tomography (CT) findings, clinical course, and outcome. We attempted to evaluate the clinical importance of failing energy metabolism and excitotoxicity as pathophysiological mechanisms in human cerebral ischemia. Such information could prove valuable in planning strategies for therapeutic intervention, for example, to combat energy failure and excitotoxicity.

Microdialysis was used in patients with aneurysmal subarachnoid hemorrhage (SAH). This disease was chosen for several reasons. It poses a considerable risk of significant secondary morbidity and mortality due to global and/or focal cerebral ischemia. The routine use of intraventricular catheters for intracranial pressure (ICP) monitoring and cerebrospinal fluid (CSF) drainage in this patient group enabled this kind of study both from practical and ethical points of view. Furthermore, the application of microdialysis could yield important information on the pathophysiology of SAH. Preliminary investigations in this area have appeared in abstract form.

Clinical Material and Methods

Patient Population

Ten patients with aneurysmal SAH were studied (eight women and two men ranging in age from 46–70 years). All patients required an intraventricular catheter for ICP monitoring and CSF drainage. The study was approved by the ethical committee at the Uppsala University Hospital and permission to use microdialysis was obtained from the patients’ relatives. The patients were treated at the neurointensive care unit and the clinical management was based on early aneurysm surgery in most cases and aggressive neurointensive care. The clinical data were collected on a computerized monitoring system. The microdialysis data were analyzed on a personal computer using commercially available software (4th Dimension; ACI-US, Inc., Cupertino, CA).

Intracerebral Microdialysis

This technique has been described in detail. Briefly, the microdialysis probe was inserted in the frontal cortex in conjunction with an intraventricular catheter used for ICP monitoring. The probe had a 4-mm polyamide membrane, and was perfused with a solution resembling CSF without glucose (140 mM Na⁺, 2.7 mM K⁺, 1.2 mM Ca²⁺, 0.9 mM Mg²⁺, and 147 mmol Cl⁻), at a rate of 2 µl/minute using a microinjection pump. An in vitro recovery test could not be done in the operating room for practical reasons owing to the sterility of the probes. As a precaution, before the patient left the operating room a microdialysis sample was routinely analyzed for lactate to reveal a malfunctioning probe, as indicated by a too-low lactate value (Table 1). Usually, 1-hour fractions were sampled, but during anesthesia, surgery, or during critical periods 10- to 15-minute fractions were collected. During the night (11 p.m.–8 a.m.) 3-hour fractions were sampled. An equilibration period of 30 to 60 minutes without sampling was allowed after probe implantation or resumption of pumping after accidental interruptions or changing of syringes. The location of the probe was determined by CT, as illustrated in Fig. 1. Microdialysis was performed over a period of 6 to 11 days. The present study is based on 1647 microdialysate samples and 16,054 chemical analyses.

Lactate and glucose were analyzed at bedside in the neurointensive care unit using an enzymatic technique (YSI 2700 Select; Yellow Springs Instruments Co. Inc., Yellow Springs, OH). The remaining samples were frozen at −80°C and later analyzed by high-performance liquid chromatography (HPLC): lactate and pyruvate by ultraviolet detection and hypoxanthine by the same method at 214 nm and 254 nm, respectively. Glutamate and aspartate were detected fluorometrically following pre-column derivatization with orthophthalaldialdehyde, by a modification of the method of Lindroth and Mopper.
To facilitate interpretation of the present results, basal dialysate levels of the measured substances, obtained in other studies with comparable microdialysis systems (membrane length 2–4 mm, perfusion rate 2 μl/min), are given in Table 1. All microdialysis results are presented as dialysate concentrations (D-) without correction for microdialysis probe recovery.

Statistical Analysis

The results were analyzed by factorial analysis of variance and by simple regression analysis using commercially available software (Stat View 4.0; Abacus Concepts Inc., Berkeley, CA). Differences with a probability value of less than 0.05 were considered statistically significant.

Results

Patient Characteristics

All patients had suffered a serious SAH. This was defined as a high Hunt and Hess grade on admission, early neurological deterioration, and/or a high Fisher, et al. grade on CT scans. Some clinical characteristics of the patients are given in Table 2. The locations of the aneurysms were as follows: four were in the anterior communicating, three in the internal carotid, two in middle cerebral, and one in the basilar artery. Nine patients underwent angiography a second time 8 to 12 days after ictus to check the aneurysm repair and to detect the presence and distribution of angiographic arterial vasospasm.

In all patients several CT scans were obtained before, during, and after the microdialysis period. A 3-month follow-up CT study was done in eight of the patients. In five patients analysis of all these imaging studies did not disclose hypodense lesions (cerebral infarcts) in the frontal lobe harboring the microdialysis probe. In four patients hypodense lesions were found in the frontal lobe harboring the microdialysis probe but not in the probe area. In one patient a cerebral infarct developed in the probe area in the frontal lobe, with the probe located within the infarct core (Fig. 1).

Clinical outcome according to the Glasgow Outcome Scale (GOS) was assessed 3 months after SAH. Four patients made a good recovery, three became moderately disabled, and three were severely disabled.

Illustrative Cases

Different patterns for the measured substances retrieved by microdialysis were observed. The patterns generally seemed to be in concert with the overall clinical course and outcome of the patients. In Figs. 2 to 4 chemical data are presented in detail from four illustrative cases.

Case 10. The microdialysis findings are shown in a patient who developed cerebral ischemia during surgery, resulting in a cerebral infarct in the frontal lobe, with the probe located within the infarct core (Fig. 1).

The patient was initially Hunt and Hess Grade IV and Fisher Grade 4. An intraventricular catheter (with the attached microdialysis probe) was immediately inserted in the right frontal lobe for CSF drainage, and the patient recovered consciousness and underwent operation on Day 3. Figure 2 illustrates the entire 188-hour (7.8 day) observation period. Prior to surgery the values of D-lactate were moderately increased compared to estimated normal values (Table 1). The D-glutamate and D-hypoxanthine levels as well as the D-L/P ratio were virtually within the nor-

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Case No. & Admis- & Aneurysm & Micro- & GOS Score & \\
& tion Grade† & Loca- & dialsysis & (3-mo & \\
& & tion & Location & Surgery & follow up) \\
& & & & Days‡ & \\
\hline
1 & II & 4 & rt ICA & lt frontal & 3–10 & 3 good recovery \\
2 & II & 4 & lt ICA & rt frontal & 1–11 & 11 good recovery \\
3 & III & 3 & lt ICA & rt frontal & 2–9 & 2 good recovery \\
4 & III & 4 & rt MCA & rt frontal & 1–8 & 2 good recovery \\
5 & III & 4 & ACoA & rt frontal & 1–9 & 3 mod disability \\
6 & III & 4 & ACoA & rt frontal & 0–8 & 3 mod disability \\
7 & IV & 4 & ACoA & rt frontal & 0–6 & 2 mod disability \\
8 & II & 2 & lt MCA & rt frontal & 3–10 & 3 sev disability \\
9 & IV & 4 & BA & rt frontal & 1–9 & sev disability \\
10 & IV & 4 & ACoA & rt frontal & 1–9 & 3 sev disability \\
\hline
\end{tabular}
\caption{Characteristics of 10 patients with SAH who underwent intracerebral microdialysis*}
\end{table}

* Abbreviations: ACoA = anterior communicating artery; BA = basilar artery; CT = computerized tomography; GOS = Glasgow Outcome Scale; ICA = internal carotid artery; MCA = middle cerebral artery; mod = moderate; SAH = subarachnoid hemorrhage; sev = severe.
† Grade on admission calculated according to Hunt and Hess classification; CT grade according to Fisher, et al.
‡ Microdialysis days refers to duration of microdialysis measurement.

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Fig. 3. Case 10. Chart showing dialysate levels of lactate, lactate/pyruvate ratio, glutamate, and hypoxanthine in conjunction with aneurysm clipping with higher time resolution compared to Fig. 2. Horizontal bars denote the periods of temporary artery clipping.

The patient had an SAH with abrupt loss of consciousness. He was intubated and ventilated and referred to us. On admission he had regained consciousness and was assessed as Hunt and Hess Grade II; his CT scan was graded as a 2 on the Fisher scale. His medical record revealed a history of congestive heart disease and pulmonary emphysema, and he was a heavy smoker. The microdialysis probe was inserted with a ventricular catheter in conjunction with the aneurysm surgery. He was extubated immediately postoperatively, but later he developed respiratory problems with hypoxemia and deteriorated neurologically with a depressed level of consciousness. He underwent reintubation and artificial ventilation.

During the initial 48-hour period, microdialysis showed markedly increased levels of D-lactate, D-L/P ratio, D-hypoxanthine, and D-glutamate (Fig. 4 upper left). The D-hypoxanthine values then decreased toward normal, whereas the concentrations of the other substances remained elevated. The D-lactate and D-glutamate concentrations showed a secondary rise starting approximately 130 hours after ictus with a sharp peak at 144 hours. This corresponded to the period of severe hypoxemia and neurological deterioration. During the final part of the microdialysis period (150–204 hours) D-glutamate remained at very high levels (> 25-fold above baseline). This coincided with an increasing trend for D-lactate, whereas the L/P ratio was stable at a slightly elevated level, and the D-glucose remained close to estimated normal values. Several CT scans failed to show an ischemic lesion in the probe area but small hypodense lesions were seen in the white matter of the right frontal lobe. At the 3-month follow-up examination, he was assessed as severely disabled on the GOS due to memory disturbances and confusion. The patient was ambulatory and had no motor deficits.

Case 5. This case also illustrates the microdialysis findings in the frontal cortex in the vicinity of a cerebral infarct. On admission the patient was assessed as a Hunt and Hess Grade III. The levels of D-lactate, the D-L/P ratio, and D-glutamate in the initial samples were increased compared to estimated normal values and showed a peak approximately 36 hours after the SAH (Fig. 4 upper right). No particular clinical event could explain this peak. A secondary increase in D-lactate, the D-L/P ratio and D-glutamate occurred approximately 80 hours after SAH and continued for approximately 2 1/2 days. During this period the D-hypoxanthine concentration curve showed several minor peaks. The D-glucose levels remained close to normal during the entire period of microdialysis. At 3-month follow up, CT scans showed bilateral hypodensities in both frontal lobes close to, but not within, the probe area. The cause of these infarcts could not be established. A repeat angiogram did not disclose arterial narrowing, but in spite of this finding, clinical vasospasm seems to be the most plausible explanation. The patient was assessed as moderately disabled on the GOS at 3-month follow-up examination.

Case 1. The chemical findings are shown (Fig. 4 lower left) in a patient with an uneventful clinical course and a good recovery according to the GOS. The patient was graded as Hunt and Hess II on admission and her CT scan was a Fisher Grade 4. An intraventricular drain was insert-
ed for ICP monitoring. Several CT scans failed to demonstrate any structural changes in the frontal lobe harboring the probe. Initially we found increased levels of D-glutamate, D-hypoxanthine, and D-glucose giving way to a moderate increase of D-lactate and D-L/P ratio during the rest of the study. The D-glutamate levels remained only slightly above estimated normal levels throughout the period of study and D-glucose fluctuated without reaching zero.

**Overall Results**

**Lactate, L/P Ratio, and Glucose.** Increased dialysate levels of lactate occurred at some point in all patients, and the concentration curves for D-lactate seemed to mirror the overall severity of the disease. However, the correlation between D-lactate levels and other indices of cerebral ischemia appeared to be relatively weak. Increased levels of D-lactate were sometimes seen without any obvious cause, and some situations of obvious energy perturbation were noted without any conspicuous rise in D-lactate, as illustrated in Fig. 3. The relationship between the mean D-lactate levels, expressed as “area under the curve,” calculated in each patient, and clinical outcome, expressed as GOS score, was analyzed. There was a tendency toward a correlation between increased D-lactate and unfavorable clinical outcome, but this correlation did not reach statistical significance (data not shown).

The D-L/P ratio seemed to reflect the ischemic state of the brain better than lactate, as demonstrated in the illustrative cases. During the early phase (0–4 days) after SAH, a statistically significant correlation between D-L/P ratio and clinical outcome was found (Fig. 5). It should be noted that an increased D-L/P ratio was sometimes also observed in frontal cortex not undergoing detectable (on CT) infarction, indicating that an increased D-L/P ratio does not necessarily signal irreversible ischemia/infarction.

**Fig. 4.** Charts showing dialysate levels of the lactate/pyruvate ratio, glutamate, lactate, glucose, and hypoxanthine. Time scales are hours after subarachnoid hemorrhage. **Upper Left:** Levels from a patient (Case 8) developing hypoxemia and multiple hypodense lesions in the brain including the frontal lobe harboring the microdialysis probe, but not in the probe area. **Upper Right:** Case 5. Microdialysates from a patient who developed a cerebral infarct in the vicinity of the probe. **Lower Left:** Case 1. Microdialysates from a patient with an uneventful clinical course and a good recovery according to the Glasgow Outcome Scale.
In most patients D-glucose levels varied between 0.2 and 1.0 mmol/L. In the patient who developed an infarct within the probe area (Case 10) D-glucose fell to zero when lactate, glutamate, and hypoxanthine accumulation was extensive (Fig. 2). In other instances when increased levels of D-lactate, D-L/P ratio, and/or D-glutamate were seen, the D-glucose levels remained above zero. In some other patients, particularly in those who had an unfavorable outcome, zero levels of D-glucose were occasionally found.

**Glutamate and Aspartate.** We found a significant correlation between the mean D-glutamate or D-aspartate levels in each patient and the clinical outcome assessed by GOS (Table 3). When studying the D-glutamate and D-aspartate levels over time in individual patients we observed that some patients had increased concentrations only early after ictus (0–4 days), whereas others had increased values only later (Days 5–7). In yet other patients increased D-glutamate and D-aspartate values were noted both early and late. To quantify the “total excitotoxic burden” of the frontal cortical tissue, the concentration curves from each patient were used to calculate the area under curve. In Fig. 5b and c these calculations are presented for both D-glutamate and D-aspartate and related to the clinical outcome expressed as GOS score. We found significantly higher area under curve values during the first 7 days after ictus in severely disabled patients than in those with good recoveries and moderate disabilities. No statistically significant difference was noted between the good recovery and moderate disability groups.

**Levels of Hypoxanthine.** In six cases increased levels of D-hypoxanthine were found during the first hours after insertion of the probe. This phenomenon, which apparently did not correlate with the patients’ clinical condition, was observed despite the fact that the first dialysis fractions were always discarded to avoid erroneous readings of the substance levels. In most cases the D-hypoxanthine levels declined toward normal and remained low. In three patients secondary elevations of D-hypoxanthine

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**TABLE 3**

<table>
<thead>
<tr>
<th>Mean glutamate and aspartate levels in 1647 microdialysate samples from 10 SAH patients (µmol/L) versus outcome*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GOS</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>glutamate</td>
</tr>
<tr>
<td>good recovery</td>
</tr>
<tr>
<td>moderate disability</td>
</tr>
<tr>
<td>severe disability</td>
</tr>
<tr>
<td>aspartate</td>
</tr>
<tr>
<td>good recovery</td>
</tr>
<tr>
<td>moderate disability</td>
</tr>
<tr>
<td>severe disability</td>
</tr>
</tbody>
</table>

*GOS = Glasgow Outcome Scale; SAH = subarachnoid hemorrhage; mean refers to average dialysate level during the entire observation period.
†Probability values less than 0.05 compared with good recovery.
were observed. These patients also showed other signs of cerebral ischemia including CT findings suggesting cerebral infarction within or close to the microdialysis probe. The highest secondary D-hypoxanthine concentrations were observed in the patient who developed an infarct within the probe area (Fig. 2). When we compared D-hypoxanthine, expressed as area under curve, and clinical outcome in all patients, a statistically significant correlation emerged (Fig. 5d).

Discussion

Microdialysis is a technique allowing the measurement of many low-molecular-weight substances in the ECF of virtually any organ. The method was described in the 1970s, and has been used extensively in experimental studies in various tissues including the brain. The first reported study in humans was published in 1987 by Lönnroth, et al., who investigated D-glucose levels in adipose tissue. The first experiences with microdialysis in the human brain were reported in 1990.

Based on experimental studies of focal brain ischemia and trauma, we have started using microdialysis with the purpose of developing it for use in neurochemical monitoring of disturbances in brain metabolism in neurointensive care patients, including those with severe head injury and SAH. The idea underlying the search for a method of continuous measurement of human brain metabolism in these acute cerebral disorders is that clinical outcome may be worsened by secondary events involving disturbed cerebral metabolism. Consequently, an improved understanding of the mechanisms of these secondary events and their early detection may open new therapeutic avenues, as well as allow a more precise use of established therapy.

In a recent study, simultaneous measurements of brain chemistry with microdialysis and measures of regional cerebral blood flow (CBF), oxygen extraction, and oxygen metabolism with PET were performed to validate the microdialysis findings with an independent method. We found agreement between the two methods; disturbances of the physiological parameters suggesting cerebral ischemia detected by PET were in general reflected by corresponding chemical changes found by microdialysis. In the present study the biochemical findings obtained by microdialysis were compared to other signs of cerebral ischemia observed on repeated CT scans and the clinical outcome assessed according to the GOS. The results lend further support to the belief that the microdialysis technique reliably detects metabolic changes in the human brain and is useful for early detection of secondary cerebral ischemia.

The changes in the ECF concentrations of energy-related metabolites observed after irreversible cerebral ischemia leading to infarction were illustrated in the patient who developed an infarct with the probe located within the infarct core. The microdialysis findings in this patient were generally in agreement with established knowledge from animal models of the biochemistry of cerebral ischemia, previously described in a number of experiments and resembled the microdialysis findings after experimental infarction in the rat. However, the absence of a robust increase of D-lactate (Figs. 2 and 3) points toward an important distinction between intracellular lactate and ECF lactate (retrieved by microdialysis) as markers of ischemia. Evidence is accumulating that lactate is translocated between the intra- and extracellular compartments with a carrier system that may be impaired during depolarization. This suggests that under conditions of energy failure (that is, severe or complete ischemia) D-lactate may not increase to the extent expected from whole-tissue measurements reflecting intracellular lactate. Such conditions would also reduce the in vivo recovery of the microdialysis probe, because of a reduced extracellular compartment, further underestimating the increase of tissue lactate. The present observation in Case 10 as well as recent experimental findings in which D-lactate increased mainly during recirculation rather than during complete ischemia in the rat and during repolarization rather than depolarization support this line of reasoning.

Increased levels of D-lactate were found in situations of energy perturbation. Although the true sensitivity and specificity could not be calculated based on this material, the findings together suggest that D-lactate is a sensitive marker of impending ischemia. However, increased levels also occurred without any obvious cause, and no statistical correlation emerged between D-lactate levels and outcome. Therefore, increased D-lactate seems to be rather unspecific as a marker of cerebral ischemia. It is well known that increased brain lactate also occurs under conditions of functional activation, low PCO\(_2\), respiratory alkalosis, and seizure activity. As mentioned before, there may also be methodological reasons for variations in D-lactate levels, such as changes in ECF volume during ischemia or edema. It should be recalled that the lactate production depends on substrate availability, and because glucose delivery depends on the arterial blood flow, complete ischemia may lead to lack of glucose in the tissue and consequently limit the lactate production. We observed that presumed events of ischemia in the patients were sometimes associated with a decrease in D-glucose to, or close to, zero, suggesting the transition from partial to complete ischemia. Because blood flow is often preserved during hypoxemia or in the vicinity of an infarct,
the dialysate glucose concentration may be used to differentiate between complete ischemia (reduced blood flow) and arterial hypoxemia or incomplete ischemia.

An increased D-L/P ratio appears to be a more reliable marker of cerebral ischemia compared to D-lactate alone. The L/P ratio may be less affected by methodological limitations than D-lactate. The present study promotes the D-L/P ratio as a useful biochemical marker of cerebral ischemia by demonstrating a statistically significant correlation between the D-L/P ratio and clinical outcome (Fig. 5a). Further support comes from the robust changes in the L/P ratio observed in Case 10 during and after temporary clipping, in which the D-L/P ratio increased dramatically whereas the D-lactate changes were less conspicuous (Figs. 2 and 3); and the patients in whom ischemic brain damage was demonstrated in the vicinity of the probe (Fig. 4 upper left and upper right). Apart from the possible methodological advantages with the D-L/P ratio, it has been shown that changes in the CSF L/P ratio closely reflect the intracellular redox state. Thus, when the ratio of the reduced form of nicotinamide-adenine dinucleotide to nicotinamide-adenine dinucleotide increased because of interrupted oxidative phosphorylation in the mitochondria (lack of oxygen), the lactate dehydrogenase reaction will be shifted towards lactate. This theoretically makes the D-L/P ratio a more specific marker of energy perturbations during ischemia, compared to lactate alone. On the other hand, in mild-to-moderate hypoxia with preserved oxidative phosphorylation, lactate and pyruvate will both increase as a consequence of increased glycolysis, with a less pronounced increase of the L/P ratio. In this situation, D-lactate will be valuable as a marker. The results of the present study appear to support these conclusions.

The rationale for using hypoxanthine was twofold. Apart from being a widely used marker of energy failure during ischemia, reflecting increased breakdown of adenine nucleotides (for example, adenosine triphosphate), hypoxanthine may be an important substrate for oxygen radical production, particularly during postischemic reperfusion. Evidence of increased free-radical production during secondary ischemia in SAH patients has recently been presented. The D-hypoxanthine findings are in agreement with our previous study. Increased D-hypoxanthine levels were found in some cases during the first several hours after insertion of the probe, also in patients with no other signs of ischemia. The reason for this finding is unclear, but these early D-hypoxanthine levels may reflect the global ischemic insult caused by aneurysm rupture. In a previous investigation we demonstrated that insertion of the microdialysis probe into the cortex elicited increased levels of virtually all substances studied. We concluded that this so-called “insertion artefact” was due to cortical injury produced by the probe and equilibration between the ECF and the dialysate. The dialysate levels returned to estimated normal levels within approximately 20 to 30 minutes and therefore all samples harvested during the initial 30 to 60 minutes after insertion of the probe are always discarded. However, it is possible that increased levels of D-hypoxanthine caused by probe insertion remain for a longer period of time than previously known and that the concentration in the early samples therefore should be interpreted with caution. A secondary rise in D-hypoxanthine was consistently seen when there were other signs of severe ischemia, and a secondary rise in D-hypoxanthine, therefore, appears to reliably reflect severe cerebral ischemia. This conclusion is further supported by the finding that increased D-hypoxanthine levels correlated to clinical outcome (Fig. 5d).

The concept of excitotoxicity is widely recognized as a potentially important mediator of acute and chronic neurodegenerative disorders. Experimental studies of focal cerebral ischemia have demonstrated that glutamate antagonists reduce the volume of the ischemic lesion in the acute phase. No such effect was found in the chronic phase of a stroke lesion in rats. The efficacy of glutamate antagonists in global or forebrain ischemia is clearly less encouraging. The importance of excitotoxicity in the development of experimental ischemic brain damage is thus still unclear and little is known about excitotoxicity in human brain injury.

The present study confirms previous reports in showing elevated glutamate concentrations in the ECF of the acutely injured human brain. We found increased levels of D-glutamate and D-aspartate in all patients after SAH, and the levels correlated to CT findings and clinical outcome. These observations support the hypothesis that excessive glutamate accumulation in the ECF and glutamate receptor overactivation play a role in the production of ischemic brain damage. An interesting observation was that increased D-glutamate was also found in the frontal cortex, which did not show infarction. This may be a neurochemical correlate to small patchy foci of cortical necrosis not seen on CT scans.

The relative contribution from different sources of the detected excitatory amino acids is unclear. Glutamate may accumulate in the cortical ECF by several mechanisms, such as release of the transmitter pool due to depolarization, reversal of the cellular reuptake system, nonspecific leakage from injured cells, and leakage via a disrupted blood-brain barrier. Possibly all sources may be involved, perhaps varying with the course of the ischemic process. Hypothetically, the early rise in D-glutamate noticed soon after temporary clipping (Fig. 3) may be caused by transmitter release, whereas at a later stage in the ischemic process, diffuse leakage from injured cells, reversed uptake, and leakage through an impaired blood-brain barrier may play a role. The source of increased glutamate found in frontal cortex not disclosing any signs of infarction on CT scans could be diffusion from injured tissue in the vicinity of the probe. With regard to glutamate receptor overactivation, excitatory amino acids from any source are likely to be important mediators of brain damage.

Another major question is what levels of glutamate are toxic. In cultured neurons as low as 2 to 5 μM of glutamate has been found to be toxic, whereas in neuron–glial cultures up to 100 μM may be needed. Using microdialysis infusion into normal rat brain, Fujisawa, et al., have estimated toxic levels to be considerably higher; approximately 20 to 30 mM. Novelli, et al., showed that toxicity of glutamate depended on the energy state of the nervous tissue and that glutamate toxicity was potentiated by low glucose or oxygen. Another possible mechanism is increased levels of glutamate that in some way interfere with membrane integrity, causing a sublethal injury that renders the neurons more vulnerable to subsequent degeneration.
Both a temporal relationship and a correlation to clinical outcome supports the concept of glutamate receptor overactivation and the present results link these events by demonstrating a threshold-type relationship between CBF and D-glutamate. The observation suggests a threshold-like relationship between energy failure and D-glutamate release. This is in line with the previously reported threshold-type relationship between CBF and D-glutamate in cat cerebral cortex.

We observed a temporal relationship between energy failure, expressed as increased D-L/P ratio, and D-glutamate accumulation after temporary clipping (Fig. 3). In our first study of human cerebral ischemia during frontal lobectomy the same phenomenon was observed. Both energy failure and glutamate toxicity are important mechanisms in the production of ischemic brain damage and the present results link these events by demonstrating both a temporal relationship and a correlation to clinical outcome.

An important consideration regarding the clinical interpretation of microdialysis data arising from the present results is that decreasing trends of energy-related metabolites may reflect quite different phenomena. Decreasing levels of D-lactate, D-L/P ratio, and D-hypoxanthine may signal a recovery following transient ischemia, but may also result from cells dying due to irreversible ischemia, as illustrated in Fig. 2. In irreversible ischemia, in which lactate production will eventually cease, the ECF levels may stay high if the ischemia is global. However, in a focal lesion within the probe area the ECF metabolites may diffuse to surrounding tissue and be taken up or metabolized by healthy cells, producing a decreasing trend in the dialysate metabolite levels. These considerations underline the point that microdialysis data must be interpreted together with other clinical information.

Unfavorable clinical outcome after SAH is to a significant extent due to mental impairment, which can include personality changes, memory deficiencies, and cognitive dysfunction, and these symptoms are mainly due to diffuse frontal lobe pathology. In this context it is important to note that we also found increased ECF levels of lactate, L/P ratio, and excitatory amino acids in noninfarcted frontal cortical tissue early after SAH. One may speculate that the frontal lobe pathology is caused by persistent lactic acidosis and moderately increased levels of D-glutamate. These mechanisms may directly damage or enhance the vulnerability of the tissue to secondary ischemia.

**Conclusions**

In conclusion, the present results support the notion that microdialysis is a promising tool for chemical monitoring of the brain in neurointensive care patients. Our results have generated a working hypothesis that the pattern of the ECF lactate, L/P ratio, glucose, hypoxanthine, and glutamate changes can be used to detect disturbances in brain metabolism and also to help differentiate between various causes of the perturbations, such as hypoxia and different degrees of ischemia (Table 4). The correlation found between the ECF levels of excitatory amino acids and outcome supports the concept of glutamate receptor overactivation in acute human brain injury.

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**References**


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**TABLE 4**

**Hypothetical patterns of extracellular substances in human cerebral ischemia and hypoxia as suggested by the microdialysis data from neurointensive care patients**

<table>
<thead>
<tr>
<th>Substance Levels</th>
<th>Ischemia</th>
<th>Hypoxia (mild/moderate)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild/Moderate</td>
<td>Severe</td>
</tr>
<tr>
<td>lactate</td>
<td>slight/mod increase</td>
<td>unchanged to slight/mod increase</td>
</tr>
<tr>
<td>lactate/pyruvate ratio</td>
<td>slight/mod increase</td>
<td>markedly increased</td>
</tr>
<tr>
<td>glucose</td>
<td>unchanged</td>
<td>decreased</td>
</tr>
<tr>
<td>hypoxanthine</td>
<td>slight/mod increase</td>
<td>markedly increased</td>
</tr>
<tr>
<td>glutamate</td>
<td>unchanged to slight/mod increase</td>
<td>markedly increased</td>
</tr>
</tbody>
</table>

* Mod = moderate.
† Hypoxia (mild/moderate) refers to hypoxemia without systemic hypotension. Severe hypoxia with hypotension would be expected to produce a pattern similar to severe ischemia.
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lar energy levels are reduced. Brain Res 451:205–212, 1988

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