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14

Microdialysis techniques in stroke research: Experimental and clinical aspects

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Abstract

Microdialysis techniques are to monitor alterations of the chemical milieu of interstitial space in the living tissue, under normal, pathological, and experimental conditions. This chapter is focusing on microdialysis studies in animal models for stroke and on clinical applications of microdialysis in patients suffering from thromboembolic stroke or from subarachnoid hemorrhage. There is substantial evidence, that microdialysis monitoring in animal models for stroke has greatly contributed to our understanding the neurochemistry of brain ischemia and can be a tool

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for testing drug candidates and novel therapeutic strategies. There are data strongly suggesting that neurochemical changes determined by microdialysis precede the onset of secondary neurological deficits following hemispheric stroke and subarachnoid hemorrhage. From the reviewed studies in patients with thromboembolic stroke it is concluded that microdialysis can be used to identify patients at risk for space-occupying edema and to select patients, who could benefit from invasive therapeutic strategies. Microdialysis monitoring of patients with subarachnoid hemorrhage revealed dramatic changes in markers for brain ischemia and specific pattern in proteomics that could be correlated to the clinical status of the patients. Cerebral microdialysis studies at bedside, however, should be designed and conducted in a way to meet the highest quality methodological standards to fulfill their diagnostic and decision-making potentials for more effective treatment of stroke patients.

Introduction

Microdialysis techniques have been developed to monitor changes in the chemistry of the extracellular space in living tissue. These techniques offer the opportunity for continuous monitoring of neurotransmitter release to various stimuli, to measure concentrations of many analytes associated with tissue damage in CNS and in various organs, to measure drug and metabolite concentrations in the interstitial space in brain and peripheral tissues and simultaneous changes in levels of targeted molecules. The microdialysis techniques require introducing an ultra thin, semi-permeable tube, a so-called probe in the tissue (Figure 1.). The tube is connected to a precision pump, which provides a steady flow of a tissue-compatible fluid through the probe at a very low flow (usually 1 – 5 $\mu\text{l}/\text{min}$). Smaller molecules in the tissue, among them the non-protein bound fraction of drug content in the extracellular fluid, will passively diffuse across the surface of the membrane and thus enter the flow of the perfusate, which is sampled at regular intervals and analyzed either on-line or off-line.

Predecessors of microdialysis probes were applied in the brain of dogs and monkey as early as 1966 and 1972 [1, 2]. Not long after, the simple hollow fiber “microdialysis probe” had been developed as described in basic reviews by Ungerstedt [3] and Chaurasia [4] on the principles, general aspects and various applications of microdialysis techniques. By now, the number of original articles on employing microdialysis exceeds 11, 000 demonstrating the usefulness of the technique.

Clinical microdialysis was introduced as intracerebral sampling method by Hillered *et al.* and Meyerson *et al.* in 1990 [5, 6]. Recent reviews have been published on the status and potential future of cerebral microdialysis, including correlations with clinical events [7, 8]. Microdialysis sampling has been performed in almost every tissue of the human body including skin [9], liver

[10], lung [11], heart [12] and peritoneum [13]. Microdialysis techniques, including transdermal/dermal microdialysis have become powerful tools for examining the pharmacokinetics and pharmacodynamics of drug candidates in preclinical as well as in clinical studies [14, 15].

This review was written from a mechanistic viewpoint to outline the use of microdialysis in monitoring cerebral ischemia in animal models and also in patients suffering from thromboembolic stroke or from subarachnoid hemorrhage (SAH).

Basic biochemical alterations in ischemic brain

Permanent ischemia

In the case of cerebral arterial thrombosis/embolism the oxygen and nutrient supply of neurons is severely reduced. If the vascular occlusion is complete the ensuing hypoxia causes a drop in ATP-synthesis, and due to the dominant glycolysis and lactic acid accumulation the pH in the brain tissue drops from 7.4 to 5 [16]. Ten minutes after the stop of blood flow the brain lactate concentration will be 5-times higher than normal, and the glucose level decreases to zero. This acidic milieu opens the acid sensitive ion-channels producing a strong Ca^{2+} -influx into the neurons. At the same time the lack of ATP causes a breakdown in the function of ion-pumps which results in K^+ efflux and Na^+ and Ca^{2+} influx. The sharply elevated intracellular Ca^{2+} - level has many consequences: a) the stored neurotransmitters will be released, which in turn amplify the disturbance, b) Ca^{2+} -dependent proteases will be activated and decompose vitally important cell proteins.

The dominant excitatory neurotransmitter is glutamate in the brain (75% of the synapses use this amino acid), and when liberated in excessive amounts it is very toxic to the neurons (excitotoxicity) [17]. The extracellular glutamate rises to an 8-fold level in 10 minutes. The ionotropic glutamate receptors (NMDA, AMPA, kainate-type ion channels) will be opened and Na^+ plus Ca^{2+} ingress can be detected. This Ca^{2+} -entry further exacerbates the process. On the other hand, the elevated intraneuronal Na^+ concentration promotes water entry and cell swelling, later osmolysis: the rupture of the neuron.

One of the earlier symptoms of ATP-depletion is the suppression of protein synthesis.

The acidosis releases iron from the stores, which metal ion converts peroxide to the very aggressive hydroxyl radical. On the other hand, the liberated glutamate blocks cystein transporters which in turn causes a decrease of the cell anti-oxidant glutathione.

A multitude of Ca^{2+} -dependent enzymes takes part in the deleterious process: nitric oxide synthase, cyclooxygenase, phospholipase-A2 and calpain-1. Calpain-1 converts xanthine dehydrogenase to xanthine oxidase.

Nitric oxide reacts with superoxide produced by xanthine oxidase and cyclooxygenase resulting in peroxynitrite which aggravates tissue damage. Phospholipase-A₂ releases arachidonic acid from the cell membrane. Cyclooxygenase later transforms arachidonate acid into prostaglandin: PGH₂. To make things worse, arachidonic acid inhibits glutamate reuptake by astrocytes, thus prolonging the high extracellular excitotoxin level. In consequence of these events, the neurons die (necrosis).

Transient ischemia

If the arterial occlusion is temporary and incomplete, then the biochemical processes are different from the formerly described ones. In this case the hypoxia and hypoglycemia also produce reactive oxygen species and some Ca²⁺ -entry into the neurons, but the key event is the consecutive egress of cytochrome-C from the mitochondria into the cytoplasm [18].

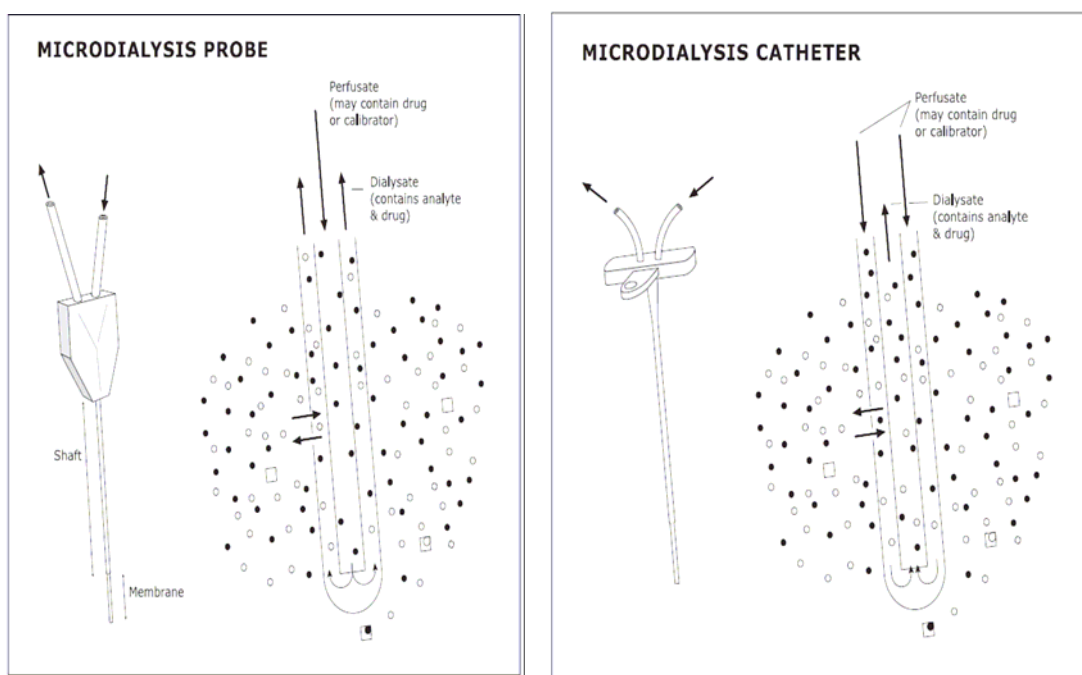


Figure 1. Schematic representation of concentric microdialysis probes for preclinical (left panel) and clinical (right panel) applications. Open circles depict the various endogenous compounds in the extracellular fluid (ECF) or in blood; the closed circles depict exogenous compounds (drugs to be tested or calibrators for determination of the *in vivo* recovery) which can be delivered by the perfusion fluid. Squares represent extracellular macromolecules that may bind analytes. In the probes for clinical application (often called catheters) the arrangement of tubes for the perfusate and for the dialysate is opposite to the arrangement in the probes for preclinical use. Dialysate is sampled at regular intervals and analyzed either on-line or off-line.

The death signals induce several genes (Fas, ERK, JNK, bcl-x, bax, TNF α , c-Jun, p53 etc.) and these in turn influence the cytochrome-C release. The liberated cytochrome-C combines with procaspase-9 and APAF-1, thus converting the enzyme to caspase-9 [18]. After this, the functional form activates procaspase-3 which degrades many proteins. Among others it inactivates DNA-repair enzymes and splits CAD-endonuclease inhibitor ICAD. In consequence DNA-fragmentation begins: this endonuclease cleaves DNA between nucleosomes.

The chain reaction ends in apoptosis but this process is much slower than necrosis.

Biochemical basis of microdialysis monitoring in ischemic brain

Microdialysis is used to determine how seriously cells are affected by ischemia, trauma, hemorrhage and various physiological, pharmacological and surgical interventions. Substantial evidence shows that *the glucose, lactate/pyruvate, glycerol and glutamate* levels are excellent markers of ischemia-induced energy failure and cell membrane damage in the brain [3, 5, 19, 20].

The brain depends almost exclusively on the aerobic consumption of glucose for energy production [21]. Brain extracellular fluid (ECF) glucose levels under normal conditions and in response to brain ischemia/hypoxia are scant [22] (Figure 2).

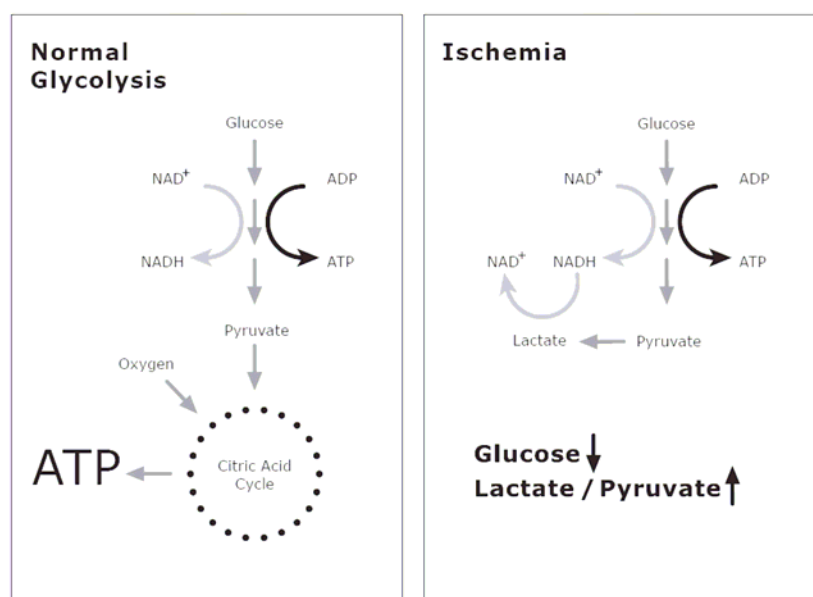


Figure 2. Normal and ischemic glycolysis.

The brain glucose concentration reflects to the balance of the oxygen supply from the blood and its utilization by cells [23]. The results of the rat ischemia model support the contention that brain glucose may be a valuable marker of severe ischemia and may help to differentiate between partial and complete ischemia [22]. In the case of complete ischemia, glucose is depleted almost totally from the brain tissue [24, 25], whereas with a lesser degree of ischemia it is decreased, but still found in the ECF (Tables 1 and 2) [26].

Table 1. High-energy phosphates and glycolysis metabolites during total ischemia in the rat. Values are means \pm standard error of mean [24].

Parameter	Control	1 minute	3 minutes	10 minutes
		Duration of Ischemia		
Glucose (mmol/kg)	4.35 \pm 0.22	0.15 \pm 0.21	0.33 \pm 0.21	0.24 \pm 0.16
Lactate (mmol/kg)	1.60 \pm 0.01	12.01 \pm 0.80	13.71 \pm 1.05	14.03 \pm 1.05
Pyruvate (μ mol/kg)	105 \pm 5	102 \pm 10	30 \pm 17	13 \pm 5
Lactate/Pyruvate ratio	15.2 \pm 0.7	123 \pm 9	1045 \pm 265	3896 \pm 2291
PCr (mmol/kg)	5.04 \pm 0.04	0.22 \pm 0.10	0.10 \pm 0.03	0.09 \pm 0.03
ATP (mmol/kg)	3.06 \pm 0.02	1.33 \pm 0.16	0.32 \pm 0.06	0.09 \pm 0.02
ADP (mmol/kg)	0.27 \pm 0.00	1.13 \pm 0.07	0.78 \pm 0.05	0.51 \pm 0.03
CO ₂ (kPa)	6.05 \pm 0.09	13.2 \pm 0.4	–	15.1 \pm 0.4
pH	7.04 \pm 0.01	6.60 \pm 0.01	–	6.48 \pm 0.01

Table 2. Brain concentrations of markers of energy metabolism and neuronal injury in patients with or without symptoms of ischemia. Values are means \pm standard error of mean [26].

Marker	Normal value	Severe Ischemia
Glucose (mmol/L)	2.12 \pm 0.15	0.54 \pm 0.15
Lactate (mmol/L)	3.05 \pm 0.32	6.73 \pm 1.09
Pyruvate (μ mol/L)	151 \pm 11.5	84.2 \pm 35.8
Lactate/glucose ratio ratio	1.62 \pm 0.18	16.7 \pm 4.7
Lactate/pyruvate ratio ratio	19.3 \pm 1.7	97.8 \pm 32.2
Glutamate (μ mol/L)	14.0 \pm 3.33	119 \pm 58.4
Glycerol (μ mol/L)	81.5 \pm 12.4	354 \pm 88.5

Several studies have shown that high-energy metabolites ATP and PCr (phosphocreatin), along with glucose and glycogen stores, are consumed during the first few minutes during normothermic ischemia, and at this time lactate has reached its maximal concentration and glucose has disappeared from the brain tissue [24]. Lactate increases during total or, in particular, partial ischemia [26]. However, the absence of a rapid increase of ECF lactate represents an important finding for distinguishing between intracellular lactate and ECF lactate [27]. Studies have shown that lactate increased mainly during recirculation rather than during complete ischemia, and during repolarisation rather than depolarization [27]. It should be recalled that the lactate production depends on substrate availability and in the case of a fast lack of glucose and pyruvate, lactate production is consequently limited (Fig. 2) [27].

Pyruvate metabolism resembles glucose metabolism. During severe ischemia its brain concentration decreases, and in the case of complete ischemia, pyruvate is used to produce lactate (Table 2) [26]. After an ischemic insult, an increase of pyruvate levels is a better marker of reperfusion than glucose levels [28].

The lactate/pyruvate ratio is a well-known marker of cell ischemia [5], and a much more reliable marker of cerebral ischemia compared to lactate or pyruvate alone [22]. The use of a ratio has the further advantage of abolishing the influence of changes in recovery over the dialysis membrane [28]. When mitochondrial function is impaired, as during anoxia or severe ischemia, the intracellular NADH/NAD⁺ ratio increases together with accumulated [H⁺] and drives the lactate dehydrogenase reaction towards lactate [22]. Pyruvate may also be consumed when α -ketoglutarate and alanine is formed from glutamate and pyruvate by alanine aminotransferase [29]. Changes in the brain lactate/pyruvate ratio appear to closely reflect the intracellular redox state [29].

The lactate/glucose ratio is also a reliable marker of ischemia [21, 30]. When cerebral oxygenation is partially reduced, lactate accumulates in the extracellular space. When severe enough, such episodes are associated with depletion of glucose from the extracellular space. This state may lead to uncompensated anaerobic glycolysis in which neurons and astrocytes compete for the extracellular glucose in a desperate bid for a trickle of adenosine triphosphate (ATP) production. In such a severe metabolic state, an increase in lactate/glucose ratio is observed, and is associated with a poor clinical outcome Table 2 [21, 30].

Neurotransmitter release as marker of ischemia and cell damage

Glutamate is released from neurons during ischemia and accumulates in the interstitial space. It is responsible for initiation of a pathological influx of calcium leading to cell damage. Glutamate concentrations have been shown to

predict the postoperative outcome of patients with subarachnoidal hemorrhage [27]. Glutamate has been shown to increase in ECF during ischemia and also in secondary ischemia [5, 31]. It is an indirect marker of cell damage, but it is sometimes difficult to interpret its changes due to the fact that the neuronally released glutamate is mixed with the large metabolic pool of glutamate [32].

Degradation of membrane phospholipids is a well-known phenomenon in acute brain injury [33]. Glycerol is an integral component of the cell membrane. Loss of energy during brain ischemia leads to an influx of calcium, which triggers events for membrane phospholipid degradation to glycerol (Fig. 3) [34]. During and after the ischemic condition, the release and production of glycerol is unbalanced. Glycerol concentrations rise during and after cerebral ischemia, and glycerol has been shown to be a sensitive and reliable marker of cell damage in experimental cerebral ischemia [33, 35].

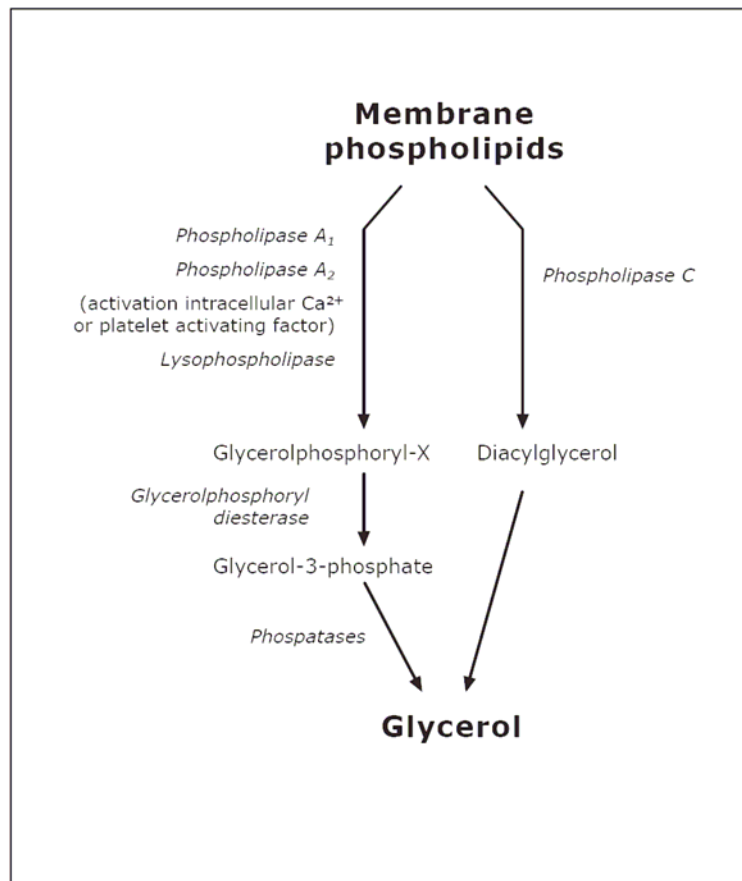


Figure 3. Biochemical pathways of membrane phospholipids degradation to glycerol modified from [35]. Phospholipids are liberated from the cell membrane through the action of phospholipases. Further degradation occurs in the cytosol. In ischemia, these processes are augmented by Ca²⁺ overload or induction of phospholipases. In the diagram, enzymes are shown in *italics*.

Microdialysis allows sampling small molecules in the brain tissue for subsequent biochemical analysis. However, in a recent paper the proteomic profile of human cerebral microdialysates was investigated [36] and the identified proteins were useful predictors for disease characteristic in stroke for tissue at risk. In samples from the stroke patients 10 proteins were found exclusively in the microdialysates not in CSF (Table 3), and it is unlikely that this is the result of tissue injury due to microdialysis probe as sampling did not begin until 18 hours after probe insertion. The appearance of these proteins could be the result of tissue compression and developing injury in the contralateral hemisphere due to expanding edema formation.

Table 3. Proteins identified solely in the cerebral microdialysates (modified from [36]).

Gene name	Protein description	Theoretical molecular weight (Da)
B2M	Beta-2-microglobulin	13823
CKB	Creatin kinase B chain	43644
DRP2	Dihydropyrimidinase related protein-2	62294
GAPD	Glyceraldehyde-3-phosphate dehydrogenase	35679
HSP73	Heat shock protein 70 kDa	70898
HBB	Hemoglobin beta chain	15867
MDHA	Maleate dehydrogenase	36295
TUBA 1	Tubulin alpha-1	50152
--	Tubulin beta	49907
TUBB5	Tubulin beta 5	49671

Experimental stroke and microdialysis

Pathomechanism of cerebral ischemia, penetration and penetration-kinetics of drugs into the ischemic brain and the effect of compounds with different mechanism of action have been studied extensively by microdialysis technique in experimental animals.

Various models of global [37, 38, 39] and focal [40, 41, 42, 43, 44, 45] cerebral ischemia, subarachnoid hemorrhage [46, 47] and concussive brain injury [48, 49, 50] were applied in different animal species (rat, monkey, cat, dog, gerbil and mouse).

In several papers the basic markers of ischemic stroke were analysed by microdialysis [40, 41, 51, 52]. Energy-related metabolites (lactate, pyruvate, glucose) were frequently monitored following cerebral ischemia [40, 41, 52, 53] in experimental animals. In a monkey study [41] continuous microdialysis and sequential PET measurements were performed simultaneously before, during and after 2 hours middle cerebral artery (MCA) occlusion. The lactate/pyruvate ratio, hypoxanthine and glutamate showed similar patterns. Probe regions with severe ischemia with or without reperfusion displayed high

and broad peaks during MCA occlusion and almost never decreased to baseline. The values in the penumbra probe regions after a slight transient increase returned to baseline. This study showed that energy metabolites and glutamate differed depending on the ischemic state and whether reperfusion occurred.

In a transient global cerebral ischemia model in gerbils [37] rapid *on-line* microdialysis coupled with liquid chromatography was developed for continuous monitoring of brain neurochemicals. Analytes were collected every 3 min over 4 hours. The perfusion flow rate was 2 $\mu\text{l}/\text{min}$. The two common carotid arteries were occluded for 10 minutes. The microdialysis probe was inserted into the striatum to simultaneously monitor pyruvate, lactate and ascorbic acid. The gerbil brain lacks the connections between the carotid and verteobasilar circulation, which makes the circle of Willis incomplete. Therefore, simple carotid artery ligation will prevent blood flow to the anterior portion of the brain. This makes the gerbil brain an excellent model for studying transient global cerebral ischemia. In this study biphasic increases in ascorbic acid and lactate/pyruvate ratio were clearly demonstrated. The rapid *on-line* microdialysis provides a near „real-time” monitoring possibility of chemical changes with the potential for investigating detailed intracranial dynamics in brain ischemia.

Marklund and coworkers [50] examined glycerol, an end product of degradation of membrane phospholipids in brain interstitial fluid by microdialysis after traumatic brain injury. The trauma caused an eightfold increase of dialysate glycerol in the injured cortex with the peak concentration in the second 10 minutes fraction after trauma. The results support the concept that extracellular glycerol, harvested by microdialysis, may be useful as a marker allowing in vivo monitoring of phospholipid breakdown.

The alterations of the extracellular concentrations of transmitter amino acids (glutamate, aspartate, glycine, γ -aminobutyric acid (GABA) and purine catabolites (hypoxanthine, inosine, adenosine) were followed for 15 hours after induction of permanent focal ischemia in cats [43]. After the transorbital occlusion of MCA dialysate samples were collected from the auditory cortex (infarct core) and from the somatosensory cortex (border zone). The results of this study indicated that excitatory amino acids are very important factors in prolonged ischemia, particularly in the border zones of ischemic foci. Excitotoxicity is enhanced by an increase of glycine and the early disappearance of adenosine, which are considered to facilitate and inhibit the deleterious effects of excitatory amino acids.

Tissue damage due to free radical activity may be a common mediator in pathology of stroke. Waterfall and coworkers studied malondialdehyde (MDA) as a marker of oxidative stress [54]. Elevation of extracellular glutamate concentrations which is detected during ischemia/reperfusion injury in the

brain increases MDA production. In a microdialysis study Yang and coworkers perfused glutamate solution or glutamate uptake transporter inhibitor into the rat brain cortex [55]. Glutamate perfusion resulted in dose-dependent increase in extracellular MDA production, while the glutamate uptake transporter inhibitor not only enhanced the glutamate level, but also dramatically increased MDA production.

MDA levels were measured not only in ischemic models, but also after experimental SAH in dogs [47]. Microdialysis catheter was inserted into the premedullary cistern. Salicylic acid was administered intravenously and microdialysate samples were analyzed for its hydroxylated products 2,3 and 2,5 dihydrobenzoic acid (DHBA). MDA was measured in subarachnoid clot removed from the prepontine cistern and in the basilar artery. There were significant correlations between basilar artery MDA levels and vasospasm and cerebrospinal fluid 2,5-DHBA levels and vasospasm. These results indicate that vasospasm after SAH is associated with lipid peroxidation.

Treatment options for stroke, a leading cause of death world-wide, are very limited. Numerous compounds reduced infarct areas in experimental stroke studies in animals, but failed in clinical trials. The animal experiments evaluate mainly infarct area and edema formation by staining methods. To improve the predictive power of experimental stroke studies microdialysis methods can be used. By continuous collection of dialysate samples from the brain (striatum, cortex and/or hippocampus) the chemical milieu can be evaluated before, during and after cerebral ischemic event.

Measurement of extracellular stroke markers by microdialysis in animals treated with putative neuroprotective compounds is a promising tool for drug development against ischemic stroke.

Zhang and co-workers used microdialysis as a tool for monitoring intracerebral glutamate release in the ischemic cortical penumbra following intravenous or intracisternal administration of DCPIB (4-(2-butyl-6,7-dichloro-2-cyclopentyl-indan-1-on-5-yl) oxobutyric acid), a volume regulated anion channel (VRAC) inhibitor [56]. When DCPIB was present in the microdialysis probe the glutamate level decreased in a statistically significant manner, infarct volume was reduced and behavioral score improved simultaneously. On the contrary, when the compound was given intravenously it failed to be neuroprotective, which means that it can not cross the blood brain barrier. This study supports the hypothesis that excitant amino acid release via VRACs contributes to ischemia-induced damage. In this case microdialysis technique helped to analyse the mode of action of this new pharmacological intervention.

The effect of non-NMDA (N-methyl-D-aspartate) antagonist GYKI-52466 on the increase of glutamate levels induced by 20 minutes four vessel occlusion (2 common carotid arteries and 2 vertebral arteries) was studied by microdialysis technique in rats [39]. In control animals, ischemia resulted in

transient increases in glutamate (4 fold), aspartate (6 fold) and GABA (15 fold) and decrease in glutamine (0.5 fold). Pre-treatment with i.v. GYKI-52466 abolished ischemia-induced glutamate release without affecting the increases in aspartate and GABA and decrease in glutamine. Post-treatment with GYKI-52466 resulted in a more rapid return of glutamate levels to basal values. In another study NMDA antagonist MK-801 and AMPA (alpha-amino-3-hydroxy-methyl-4-isoxazoly-propionic-acid)/kainate antagonist NBQX molecules showed different effects on glutamate and aspartate levels in a similar forebrain ischemia model in rats [57]. The NMDA receptor antagonist inhibited the ischemia-induced accumulations of excitant amino acids while the AMPA antagonist compound did not alter the neurotransmitter levels during ischemia. On the contrary, a novel AMPA antagonist 2,3 benzodiazepine which is the structural analogue of GYKI-52466 was found to be neuroprotective in transient focal ischemia in acute [58] and chronic [59] experiments in rats. Its effect on cerebral glutamate liberation has been studied in our laboratory by microdialysis. It reduced the ischemia-induced striatal glutamate release mainly during the reperfusion period given 30 minutes prior to MCA occlusion (unpublished data).

A recent paper demonstrated by microdialysis technique that glutamate release is inhibited by granulocyte-colony stimulating factor after experimental stroke in rats [60]. It also was described that red wine polyphenol compounds mediate neuroprotection in focal brain ischemia. Extracellular amino acids and energy metabolites were measured and proteomic analysis was applied from brain microdialysis samples in rats [61]. The classical markers of brain damage (infarct volume, regional cerebral blood flow) well correlated with changes of the chemical milieu in the ischemic hemisphere.

Clinical applications of cerebral microdialysis in stroke

Microdialysis in human brain was performed for the first time in 1989 in patients with Parkinson's disease, who underwent thalamotomy [6]. The aim of that study was to test the reliability of the microdialysis sampling for biochemical characterization of a target area in the human brain during a routine operation. By now the number of articles on cerebral microdialysis in human brain exceeds 8000. As it was predicted [20], monitoring neurochemical changes and drug penetration by cerebral microdialysis has become routine clinical technique in several countries [for recent reviews see: 8, 15, 62, 63, 64]. This section will focus on the application of microdialysis in patients suffering from thromboembolic stroke or from subarachnoid hemorrhage.

Thromboembolic stroke

Ischemic stroke in MCA territory often leads to malignant brain infarcts representing an 80 % mortality risk with conservative treatment [65, 66]. Therefore nonconservative treatment strategies, such as induced hypothermia [67, 68] and decompressive hemicraniectomy [69, 70] might be justified as they may improve clinical outcome of patients with severe space-occupying stroke. The time course of pathobiochemical changes (*see previous sections of this chapter and chapters 6,9,12,13*) characteristic for ischemic brain can be monitored by cerebral microdialysis. Neurochemical monitoring by microdialysis in combination with determination of intracranial pressure probe (ICP) and with various imaging techniques can identify predictors of a malignant course and to determine the time point of critical deterioration in patients with large MCA infarctions [71].

In a case report of a 43-year-old patient suffering from left hemispheric stroke with a space-occupying postischemic edema, a massive increase in levels of glutamate, glycerin, and the lactate-pyruvate ratio was measured 24 hours before intracranial pressure elevation was observed and brain death occurred [72]. The microdialysis and ICP probes were placed into the noninfarcted hemisphere. To assess potential predictors of malignant brain edema by measurement ICP and microdialysis in 10 patients with large hemispheric stroke and different clinical courses [73]. ICP and microdialysis probe were placed into the parenchyma of the ipsilateral frontal lobe of the patients. Extracellular concentrations of glutamate, lactate, pyruvate, and glycerol were measured continuously. Repeated cranial CT scans were used to determine the size of infarction and presence of mass effect. The dynamics of the different neurochemical markers varied in harmony with the clinical course, size of infarction, and local brain edema. Increase in ICP and in glutamate concentration and lactate-pyruvate ratio was followed by massive edema and large infarcts; generally low and stable ICP and substrate concentrations were found in patients without progressive space-occupying infarct. In a larger scale study [74] 50 patients had been included who required critical care due to massive stroke of the MCA territory. By correlating the microdialysis results with follow-up CT scans, the neurochemical characteristics of three different brain compartments were defined: (1) noninfarcted brain tissue with normal microdialysis values, (2) brain areas adjacent to the infarct core which were not hypodense in CT scans but caused reversible neurochemical alterations, and (3) the infarct core with massive concentration changes which did not normalize over the measuring period of 3 to 5 days. Microdialysis values correlated with initial PET scans helped to describe neurochemical predictors of a malignant, i.e., life-threatening, space-occupying course of the ischemic stroke.

Cerebral blood flow (CBF) and extent of irreversible tissue damage as well as the time course of extracellular concentration of transporter amino acids, substrates of energy metabolism, and purine metabolites, ICP and tissue oxygen tension were assessed in 34 patients with large strokes of more than 50% of the MCA territory [75]. Development of malignant brain infarcts was predicted by the size of critically hypoperfused tissue and the volume of irreversibly damaged tissue. The course of malignant infarcts was characterized by progressive increase in concentrations of excitatory amino acids, lactate, pyruvate, glycerol, and hypoxanthine and in ICP, while cerebral perfusion pressure (CPP) and tissue oxygen tension decreased. These results clearly differentiate a malignant from a benign course of large hemispheric infarction. The results of these authors suggest that their methods can be used to identify patients at risk for formation of space occupying edema and to select patients who could benefit from invasive therapeutic strategies.

In a more recent work from the same group [76] multimodal neuromonitoring including measurement of CPP, tissue oxygen pressure, and microdialysis was used to study cerebrovascular autoregulation and its impact on clinical course in 15 patients with malignant MCA infarction. Probes were placed into the ipsilateral frontal lobe. Autoregulation was assessed by calculation of the CPP-oxygen reactivity index (COR) and the correlation coefficient (R) of CPP and tissue oxygen pressure at 24 and 72 hours after stroke. COR and R at 24 hours after stroke were higher in the 8 patients with a malignant course compared with the 7 patients with a benign course, indicating impaired autoregulation in the malignant course group. At 72 hours, further increases in COR and R were observed in the malignant course group in contrast to the benign course group with stable values over time. The lactate:pyruvate ratio was higher in patients with a malignant compared with a benign course at both time points. COR, R, and the lactate-pyruvate ratio showed significant correlations with outcome parameters.

Shimada and coworkers reported alterations of both the extracellular transmitter and non-transmitter amino acids in a cat ischemia model [77]. Microdialysis was used to determine alterations of extracellular non-transmitter amino acids for the first time in human stroke as a potential early marker for expansion of extracellular space [78]. Thirty-one patients with infarctions covering >50% of the MCA territory in early cranial CT scans were included in the study. Probes for microdialysis, ICP, and tissue oxygen pressure were placed into the noninfarcted ipsilateral frontal lobe. PET imaging was performed in 16 of these patients to measure CBF in the tissue around the neuromonitoring probes. Fourteen of the 31 patients developed a malignant MCA infarction, and 17 did not. The patients in the malignant group had significantly lower extracellular concentrations of non-transmitter amino acids than those in the benign group in the first 12 hours of neuromonitoring.

At this time, CBF values determined in regions of interest around the probes by positron emission tomography and tissue oxygen pressure showed that the monitored tissues were not yet infarcted, and no differences in transmitter amino acids concentrations were found between the 2 groups. Furthermore, extracellular concentrations of non-transmitter amino acids were negatively correlated with the size of infarction.

In addition to monitoring the neurochemical changes in ischemic brain, microdialysis has been used to assess the effect of various treatments of patients with malignant MCA infarction. Neurochemical changes were monitored in the extracellular space in a patient with left-sided hemispheric infarction treated with moderate (33 °C) hypothermia [79]. Microdialysis samples were obtained from the infarcted and noninfarcted hemisphere during hypothermia and rewarming. Levels of ischemic markers in the infarcted hemisphere (glutamate, glycerin, lactate/pyruvate) decreased with hypothermia and remained stable (glutamate) or increased (glycerin, lactate/pyruvate) during rewarming. Concentrations of these substances in the noninfarcted hemisphere remained at normal levels. Microdialysis monitoring of therapeutic hypothermia in severe hemispheric infarction might be a useful additional monitoring tool to assess the status of the brain and to predict further deterioration. In another study [80] from the same group the authors assessed the effect of therapeutic moderate hypothermia on excitatory amino acids and metabolism in 12 patients suffering from space-occupying MCA infarction. In an open, prospective, observational study the patients were undergoing moderate hypothermia (33 °C) as rescue therapy for large, life-threatening middle cerebral artery infarction. Microdialysis probes were placed concomitantly with ICP measuring devices in the frontal lobe of the infarcted and/or noninfarcted hemisphere. According to follow-up cranial CT scans, 3 different compartments of microdialysis measurements could be defined. First, noninfarcted brain tissue had stable dialysate concentrations but a significant effect of hypothermia on glutamate (2.6 versus 3.6 micromol/L), lactate (1.8 versus 3 mmol/L), and pyruvate (50 versus 95.8 micromol/L) was found. Second, measurements from peri-infarct tissue had a significant effect of hypothermia on glutamate (4.8 versus 12.6 micromol/L), glycerol (58 versus 82 micromol/L), lactate (0.7 versus 1.3 mmol/L), and pyruvate (13.3 versus 36.8 micromol/L). Third, dialysate concentrations obtained from irreversibly damaged tissue were excessive for glutamate (453 micromol/L), glycerol (1187 micromol/L), lactate (12 micromol/L), and pyruvate (4 micromol/L). In this extreme compartment hypothermia had no effect. These results suggest that hypothermia decreases glutamate, glycerol, lactate, and pyruvate in the "tissue at risk" area of the infarct, but not within the infarct core. Thus, future treatment strategies for life-threatening stroke should be guided by close neurochemical monitoring.

Orally applied glycerol as osmotic agent was used to reduce increased ICP [69]. Glycerol had also been used intravenously to treat brain edema in stroke patients [81, 82]. In a recent study the pharmacokinetics of glycerol was investigated in brain tissue by cerebral microdialysis in patients with large MCA infarction [66]. The following variables were assessed after intravenous administration of 25 g of glycerol before and every 10 minutes until 80 minutes: ICP, serum osmolarity, and cerebral microdialysate concentrations of glycerol, glutamate, pyruvate, and lactate. During 16 ICP crises in 7 patients, cerebral glycerol concentrations increased immediately after glycerol administration by up to 350%. Conversely, ICP rapidly decreased by almost 50%. Both effects lasted for 70 minutes. Serum osmolarity was only briefly raised, whereas glutamate, lactate, and pyruvate remained unaffected.

Fatty acid ethanolamides (FAEs) may be generated in brain neurons after excitotoxic insults and they have neuroprotective effects associated with their ability to inhibit glutaminergic neurotransmission [83, 84, 85]. Release of FAEs such as anandamide, palmitylethanolamide (PEA), and oleylethanolamide (OEA) was studied for the first time in the brain of a patient suffering from malignant hemispheric infarction treated with hypothermia [86]. A patient with life-threatening hemispheric stroke was treated with moderate hypothermia (33 °C) that was maintained for 3 days, followed by a 3-day rewarming period. Microdialysis was applied to measure glutamate, lactate, and glycerol by using a microdialysis analyzer. FAEs were measured by microdialysis coupled with high-performance liquid chromatography/mass spectrometry. Release of neuroprotective fatty amides occurred within the first day after ischemia and reached high concentrations for all three substances in tissue surrounding the primary ischemic lesion: anandamide up to 42 pmol/mL, PEA up to 120 pmol/mL, and OEA up to 242 pmol/mL. There was a significant correlation with the elevation of lactate as early marker for the hypoxic insult.

In most of the cases cerebral microdialysis has been used to monitor changes of concentration substances of low molecular weight in the ECF of patients suffering from severe stroke. In a seminal paper by Maurer and coworkers [36] the proteomic profile of human cerebral microdialysate was determined to elucidate if the identified proteins might be useful predictors for disease characteristics in stroke for tissue at risk in the contralateral hemisphere. It has been published, that ischemia produces a global down-regulation of protein synthesis in the brain [87, 88, 89], but the expressions of some proteins are upregulated in both the ischemic and the contralateral hemisphere after hypoxia [90, 91]. Using a proteomic approach based on two-dimensional gel electrophoresis and subsequent mass spectrometry, Maurer and coworkers created a protein map for the global protein expression pattern of human microdialysate. They found an average of 158 ± 24 (N = 18) protein

spots in the human cerebral microdialysate and could identify 95 spots, representing 27 individual proteins. Most of these proteins had been detected in human CSF before, but 10 additional proteins mainly of cerebral intracellular origin were identified only in the dialysate samples. The correlation of protein expression in the human cerebral microdialysate with the patients' clinical condition and results of cerebral imaging may be a useful approach to future applications for neurological stroke diagnosis, prognosis, and treatment.

Subarachnoid hemorrhage

SAH is bleeding into the subarachnoid space, the area situated between the arachnoid membrane and the pia mater. SAH causes between 1 and 7% of all strokes [for recent reviews see: 92, 93]. Spontaneous SAH is most often due to rupture of cerebral aneurysms (85%), or weaknesses in the wall of the arteries of the brain that become enlarged. In 15–20% of cases of spontaneous SAH, no aneurysm is detected from the first angiogram [94]. The exact mechanism(s) by which SAH induces arterial vasospasm is still subject of considerable research and debate [95]. Regardless of the cause, it is regarded as a medical emergency. Cerebral vasospasm remains a significant source of morbidity and mortality in patients with SAH after an aneurysmal rupture [96]. Arterial vasospasm typically appears 3 to 4 days after rupture and reaches a peak in incidence and severity at 7- 10 days. The incidence and time course of symptomatic vasospasm parallels that of arterial vasospasm. However, while 40% to 70% of patients have evidence of arterial narrowing (angiography or Doppler ultrasound), only 20% to 30% develop the clinical syndrome [92]. Symptomatic vasospasm typically begins 4-5 days after the hemorrhage. When the arterial narrowing is marked, these symptoms may progress to focal neurological deficits, infarction, coma and death. In less severe cases, neurological recovery can be expected as the arterial narrowing resolves.

Microdialysis in combination with determination of tissue oxygenation and with various imaging techniques has been used in search for early indicators of secondary deterioration in patients with SAH. Introduction of microdialysis technique into many European neurointensive care units to monitor brain chemistry in patients has resulted in variations in the application methods. In a methodological paper authored by Hutchinson et al. [97] several variables, including length of the catheter membrane, type of perfusion fluid, flow rate of perfusion fluid, and on-line compared with delayed analysis of samples were considered. Variations in perfusion fluid and freezing and thawing of samples did not result in differences in substance concentration. Catheter length had a significant impact on substance recovery. The recovery was approximately 70% at 0.3 μ l/minute and 30% at 1 μ l /minute (10-mm membrane)

for all analytes. Glutamate results obtained with the enzyme analyzer showed good correlation with those from HPLC.

At a meeting in Stockholm a consensus agreement was achieved by a group of experienced users of microdialysis in neurointensive care, defining the use of microdialysis, placement of catheters, unreliable values, chemical markers, and clinical use in SAH and also in traumatic brain injury (TBI) patients [98]. The participants of the meeting recommended the following procedures for using microdialysis in SAH: (i) microdialysis should be used in severe cases needing monitoring of ICP and CPP; (ii) probes/catheters should be placed in the tissue at risk – most likely the parent vessel territory; (iii) values from a period of at least 1 h after the insertion should be considered unreliable; (iv) Lactate/pyruvate ratio and glutamate are sensitive markers for the development of brain ischemia (lactate alone is a insufficient marker).

Microdialysis technique to monitor changes in ischemic brain was applied in a patient with severe SAH for the first time in 1992 [28]. A microdialysis probe was inserted into the right frontal probe and concentrations of the energy-related metabolites lactate, pyruvate, and hypoxanthine were measured, and the lactate:pyruvate ratio was calculated. In addition, the acids glutamate, aspartate, taurine, glutamine, asparagine, and glycine were measured. The initial lactate levels were very high and the lactate:pyruvate ratio fluctuated at a high level, but dropped later as CSF was drained. The hypoxanthine levels, marker for increased ATP degradation were also raised initially and fell later. The pattern suggests profound disturbance in cortical metabolism during the first 10 – 15 hours after starting of microdialysis. Concentrations of amino acids showed three different patterns: (i) glutamate, aspartate and taurine levels were about 25 times higher compared to normal levels during the first hours and then decreased considerably; (ii) glutamine level was stable; (iii) levels of asparagine and glycine tended to increase with time.

Concentrations of the energy-related metabolites lactate, pyruvate, and hypoxanthine of ECF were determined in all the subsequent microdialysis studies in patients with SAH reviewed by us [26,27,33,99,100,101,102,103,104,105,106] and most of them also monitored changes in glutamate levels of the ECF. There is evidence from animal experiments [50] that glycerol is a marker for post-traumatic membrane phospholipid degradation. Increased levels of ECF glycerol have been found in SAH patients [33,100,105,106] similarly to patients suffering from MCA occlusion [80] and from TBI [107]. In a patient with a stable condition and without signs of secondary hypoxia/ischemia, the glycerol concentration remained low. Simultaneous determination of glycerol in arterial plasma samples showed that the changes in brain interstitial glycerol could not be attributed to systemic changes and an injured blood brain barrier [33]. Bedside intracerebral microdialysis monitoring of ten patients with SAH and signs of

delayed ischemia revealed pronounced changes in concentrations of glucose, lactate, and glycerol in ECF that could be directly correlated to the clinical status of the patients. Patients with uneventful clinical courses generally demonstrated low and stable levels of the different metabolites, and those with signs of cerebral ischemia demonstrated various patterns of neurochemical changes. Lactate and glutamate seemed to be sensitive markers of impending ischemia, and increased glycerol levels were associated with severe ischemic deficits [100] in line with earlier [33] and later [26] findings.

In recent studies [105,106] microdialysis and PET was compared in the detection of ischemia in SAH patients. Extracellular glucose, lactate, lactate/pyruvate (L/P) ratio, glutamate, and glycerol levels were analyzed hourly in the brain in thirteen SAH patients. Regional CBF (rCBF) was determined in the volume of interest of the microdialysis catheter and all vascular territories. Microdialysis values were correlated to rCBF on the day of PET. Then, dialysate concentrations of asymptomatic versus ischemic phases were analyzed. In symptomatic patients (n=10), rCBF was significantly lower compared with controls (n=3, P=0.048). Glutamate correlated best with rCBF (r=-0.66; P=0.014), followed by glycerol (r=-0.62; P=0.021). The L/P ratio was most sensitive (0.82) and specific (1.0) in indicating symptoms of ischemia, but only during longer periods of ischemia [105]. In another study of the same group [106], microdialysis catheter was inserted into the brain parenchyma most likely to be affected by vasospasm directly after aneurysm clipping. Glucose, pyruvate, lactate, glutamate and glycerol were analyzed hourly (CMA 600). 15O-H₂O-PET scans and 18F-FDG-PET scans were performed between days 2 and 17 after SAH. Regions of glucose hypometabolism were observed in 10 patients with symptoms of ischemia. Their rCBF was lower compared to asymptomatic patients (p<0.05). Dialysate levels of glutamate, lactate, the L/P ratio and glycerol were significantly higher in symptomatic than asymptomatic patients (p<0.05). Microdialysis parameters were well correlated with glucose hypometabolism (18F-FDG-PET) and symptoms of ischemia. However, the threshold for a metabolic derangement was above the limits of cerebral ischemia defined by PET.

In addition to the increase of lactate, lactate:pyruvate ratio and increase of excitatory amino acids (EAA) in ECF as most sensitive markers for cellular imbalance in ischemic brain, alterations of concentrations of other substances in dialysates also can be predictive for unfavorable outcomes of patients suffering from SAH. A study authored by Staub and coworkers was conducted to search for putative indicators of primary and secondary brain damage in patients with aneurysmal SAH [101]. Microdialysis probes were placed, with a ventriculostomy catheter for drainage of cerebrospinal fluid, into a frontal lobe of 10 patients for 4.6 ± 0.5 days. Amino acids, metabolites of glycolysis, purines, catecholamines, and nitric oxide oxidation byproducts were measured

in the dialysates. When peak concentrations in the dialysates of patients with favorable and unfavorable outcomes were compared, significantly higher levels of excitatory amino acids, taurine, lactate, and nitrite, but not of purines and catecholamines, were observed for patients with poor outcomes ($P < 0.05$). (However, nitrate levels did not show profound differences.) The results of the study confirm the usefulness of EAAs and lactate as major parameters for neurochemical monitoring for patients threatened by acute cerebral disorders. Other substances, such as taurine and nitrite, were also demonstrated to be potentially predictive. In contrast to these results, decrease in nitrite and nitrate levels was found regardless of development of delayed ischemia [108]. In another study with microdialysis monitoring in the cortex of patients with severe SAH who were at risk for developing secondary brain damage and vasospasm, NO metabolites, glucose, and lactate were analyzed to determine the time course of NO metabolite changes and to test the interrelationship between the analytes and clinical variables [109]. No significant correlation between NO-production, brain tissue carbon dioxide tension, and dialysate glucose and lactate was observed. But brain tissue oxygen tension was strongly correlated to dialysate nitrate and nitrite ($r^2 = 0.326$; $P < 0.001$); however, no correlation was noted between brain tissue oxygen tension and NO-metabolites in CSF ($r^2 = 0.018$; $P = 0.734$). The authors of that study suggest that substrate delivery and NO are linked in the pathophysiology of vasospasm after SAH.

Increases in the extracellular concentration of the EAAs, glutamate and aspartate during cerebral ischemia in SAH patients are well recognized. Less emphasis has been placed on the concentrations of the inhibitory amino acid neurotransmitters, notably GABA, despite evidence from animal studies that GABA-potentiating drugs may act as a neuroprotectant in models of ischemia [110]. The objective of a study [111] was to investigate the concentrations of various excitatory, inhibitory and non-transmitter amino acids under basal conditions and during periods of cerebral ischemia in patients with head injury ($n=7$) or with SAH ($n=5$). Patients were monitored in neurointensive care or during aneurysm clipping. In six patients, cerebral ischemia was associated with dramatic increases in the concentration of GABA, in addition to the glutamate and aspartate. Parallel increases in the concentration of glutamate and GABA were found ($r=0.71$, $p<0.005$) indicating that cerebral ischemia is not accompanied by an imbalance between excitatory and inhibitory amino acids. In fact, the results raise the possibility of an endogenous GABA mediated neuroprotective mechanism in humans.

A major complication of aneurysmal SAH is symptomatic vasospasm, a complex syndrome consisting of neurological deterioration and exclusion of other sources of ischemia. Approximately 30% of SAH patients are affected [96]. Although symptomatic vasospasm is associated with high mortality and

poor clinical outcome, it is not possible to identify the individual risk on a molecular level for patients before symptoms have developed. In a recent study [112], the authors hypothesized that protein changes occur in the cerebral microdialysate of patients who later develop symptomatic vasospasm which are not found in matched-pairs control subjects. They searched for changes in protein concentrations in microdialysate sampled from the fronto-temporal brain tissue of five vasospastic and five nonvasospastic SAH patients using proteomics technology based on two-dimensional gel electrophoresis and mass spectrometry. Microdialysate samples were taken at least 1.5 days before the onset of symptomatic vasospasm. Comparing protein expression profiles, it was found that the protein concentrations of several isoforms of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were 1.79-fold \pm 1.29 (N=5, P<0.05) higher in the group which later developed symptomatic vasospasm, whereas heat-shock cognate 71 kDa protein (HSP7C) isoforms were decreased to 0.50-fold \pm 0.19 (N=5, P<0.05; all expression data means \pm s.d.). The changes in protein concentrations were detected 3.8 \pm 1.7 days (N=5, P<0.05) before symptomatic vasospasm developed. It is concluded that GAPDH and HSP7C may be used as early markers indicating the later development of symptomatic vasospasm after SAH, enabling selective early therapeutic intervention in this high-risk group of patients.

Advantages and limitations of cerebral microdialysis in stroke

For searching markers of primary and secondary brain damage, cerebral microdialysis is particularly well suited because time course with long observation period can be combined with determinations of multiple substances with putative indicator function. Microdialysis is the only technique available for cerebral metabolic monitoring in the clinical setting. In addition, it permits the assessment of metabolic changes after therapeutic interventions. Unlike to CSF sampling and PET, microdialysis catheter placed in the tissue monitors the local biochemistry. In contrast to other sampling methods of biological fluids and tissues, microdialysis sampling provides a very clean dialysate with normally no need for cleanup procedure. Earlier the inadequate sensitivity of bioanalytical methods represented a major limitation of microdialysis techniques. By now, the analytical methods are sensitive enough to determine the substance of interest at very concentrations. Monitoring only very small volume of tissue surrounding the catheter/probe offers both advantages and disadvantages. On one hand, catheter can be placed in the tissue at risk; the vascular territory most likely to be affected by vasospasm or the penumbra surrounding a lesion. In such locations microdialysis offers an early warning of impending ischemia, and by this way it may guide therapeutic intervention. On the other hand, unintentional placing of a catheter in normal tissue may result in a delay in the indication of ischemia.

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