Increased cerebral lactate levels are a well-known aspect of the sequelae of the metabolic derangements that follow cerebral ischemia. A new technique has recently become available to sample cerebral venous blood from the superior sagittal sinus on a long-term basis in conscious rats. We report the applicability of this method to assess serial biochemical responses to brain injury. Serum samples were obtained from the superior sagittal sinus, the common carotid artery, and the external jugular vein of nine anesthetized rats before and up to 7 days after 10 minutes of forebrain ischemia was produced by carotid occlusion and hypovolemic hypotension (mean arterial blood pressure 50±4 mm Hg). The cerebral venous—arterial difference in serum lactate concentration was increased for up to 3 hours after ischemia, while there was no significant change in the difference in serum lactate concentrations in the common carotid artery and the external jugular vein. This indicates an elevated output of lactate from brain tissue to blood, detectable only in the superior sagittal sinus, which underlines the usefulness of the technique. We observed a persistent elevation in brain lactate production after virtually complete recovery from the acute insult. (Stroke 1990;21:614–617)

Recently we demonstrated the feasibility and benignity of a novel technique for sampling cerebral venous blood in conscious rats on a long-term basis. This method permits blood from the superior sagittal sinus to be repeatedly sampled for 7 days. To investigate the applicability of this technique for studies of cerebral metabolism, the cerebral output of LA was assessed by measuring LA concentrations in serum from different sources, such as the superior sagittal sinus, the common carotid artery, and the external jugular vein, in a model of partial global ischemia. The ability to obtain serial samples on a chronic basis has permitted analysis of the dynamics of LA release from brain to cerebral venous blood for up to 7 days after partial global ischemia in rats.

Materials and Methods

We used nine barrier-reared male Sprague-Dawley rats (Taconic Farms, Inc., Germantown, New York) weighing 350–400 g that had free access to food and water. Anesthesia was induced with 12 mg i.p. ketamine+0.12 mg i.p. acepromazine/100 g body wt. Both femoral arteries were cannulated with PE 50 tubing for monitoring of mean arterial blood pressure (MABP) and withdrawal of blood. Following depilation of the scalp and the upper back, the rats were placed in a stereotactic frame (David Kopf Instruments, Tujunga, California). A catheter system
for chronic sampling of cerebral venous blood was implanted into the superior sagittal sinus as described previously. In brief, a 24-gauge Teflon catheter was inserted into the superior sagittal sinus, which was perfused with a nontoxic dose of heparin solution (1 IU heparin/hr) by a miniosmotic pump (model 2001, Alza Corp., Palo Alto, California). Cerebral venous blood could be obtained through a Y-connection.

Following implantation of the superior sagittal sinus catheter, the rats were removed from the stereotactic frame and placed in the supine position. Both common carotid arteries were exposed through an incision in the midline of the neck. The left common carotid artery was cannulated in an anterograde fashion, and the PE 50 tubing was exteriorized on the backs of six rats for sampling of arterial blood. In the three other rats, in addition to the preparation above, a PE 50 catheter was also inserted into the right external jugular vein up to 3 hours after ischemia for sampling of venous blood to evaluate the specificity of the superior sagittal sinus as a source of metabolites released into the cerebral venous blood.

Forebrain ischemia was induced in accordance with the method of Smith et al. Systemic hypotension was induced by controlled withdrawal of arterial blood from the femoral line. As soon as MABP reached 50 mm Hg, the right common carotid artery was clamped with an aneurysm clip (50 g) for 10 minutes while the MABP was maintained at 50±4 mmHg (mean±SEM) by infusion or withdrawal of blood as needed. The shed blood was kept in heparinized syringes at room temperature during ischemia. Reperfusion was achieved by removal of the aneurysm clip and infusion of the shed blood over 4 minutes. Carotid blood flow was confirmed by direct visualization. The neck incision was then approximated with 4-0 silk, and the rats were allowed to recover. During surgery and recovery, body temperature was maintained at 37.5°C by a heating pad.

Blood samples (0.1 ml whole blood per sample) for measurements of serum LA in the superior sagittal sinus, common carotid artery, and external jugular vein were obtained 10 min before and 2, 15, 60, 120, and 180 minutes, 24 hours, and 7 days after 10 minutes of forebrain ischemia. Samples from all sources were withdrawn at a constant rate (0.5 ml/min) and immediately centrifuged at 1,600 rpm (Microfuge B, Beckman Instruments, Inc., Fullerton, California) for 1 minute. The serum was rapidly decanted and kept in dry ice. The entire sampling procedure required 2 minutes. Duplicate serum samples (20 µl) were used for LA assay with a semiautomatic industrial lactate analyzer (model 27 and 2746 L-Lactate Kit, Yellow Springs Instrument Co., Yellow Springs, Ohio) that was calibrated and tested for linearity with two LA standards (50 and 150 mg%) every 20 measurements.

The experiments were conducted according to the principles set forth in the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Resources, National Research Council (Department of Health, Education, and Welfare Publication No. NIH 85-23, 1985).

All data are expressed as mean±SEM. Changes in serum LA concentrations were tested using the Kruskal-Wallis analysis of variance followed by Mann-Whitney U test. All data were expressed as mean±SEM. Changes in serum LA concentrations were tested using the Kruskal-Wallis analysis of variance followed by the Mann-Whitney U test; p<0.05 was accepted as the level of significance.

Results
All rats recovered from surgery and ischemia. Patency of the sinus catheter was preserved for the entire 7 days. Blood samples from the common carotid artery could be obtained without flushing the line for 24 hours after ischemia.

Figure 1 demonstrates the LA concentration in arterial blood of six rats before and after 10 minutes of forebrain ischemia; LA concentration in the common carotid artery (LA_CCA) was significantly greater than the control level shortly after ischemia (fourfold increase to 26.36±2.72 mg%, p<0.01). LA_CCA was normalized by 15 minutes after ischemia and showed no more significant changes later, including at 24 hours (LA_CCA=7.83±1.39 mg%), when rats had fully recovered from anesthesia and surgery. Control LA levels in our study were comparable to those in other species. Under control conditions, the concentration of LA in the superior sagittal sinus (LA_SSS) was slightly higher than that in arterial blood (LA_SSS−LA_CCA=1.49±0.09 mg%, p<0.01, n=6).

Postischemic LA dynamics in cerebral venous blood from the superior sagittal sinus did not parallel those in the arterial blood. Figure 2 demonstrates the cerebral venous—arterial differences in LA concentration (LA_SSS−LA_CCA) for six rats at each time. The difference was increased significantly greater than the control level shortly after forebrain ischemia (5.97±0.56 mg%, p<0.01). By 120 minutes after forebrain ischemia, the difference was still significantly greater than control, but it declined further and was no longer significantly different from control.

FIGURE 1. Graph of mean±SEM lactate concentration in common carotid artery (mg%) before and after 10 minutes of forebrain ischemia in six rats. *p<0.01 different from control by Kruskal-Wallis analysis of variance followed by Mann-Whitney U test.

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FIGURE 2. Graph of mean±SEM cerebral venous—arterial difference in lactate concentration after 10 minutes of forebrain ischemia in six rats. Differences calculated as concentration in blood from superior sagittal sinus minus that in blood from common carotid artery. * **p<0.05, <0.01, respectively, different from control by Kruskal-Wallis analysis of variance followed by Mann-Whitney U test.

by 180 minutes after ischemia. By 24 hours, the difference had completely normalized. By 7 days, only cerebral venous blood from the superior sagittal sinus could be obtained, and LASS (8.20±1.3 mg%) was not significantly different from LASSS under control conditions (8.43±1.03 mg%).

Figure 3 demonstrates the differences between LASS and LA concentrations in the external jugular vein (LAEJV) and LACCA, in three rats. Control LAEV (7.74±1.2 mg%) was not significantly different from control LASS (8.43±1.03 mg%). Shortly after forebrain ischemia, LASS and LAEV showed concurrent changes but dissociated significantly at all later sampling times (p<0.05). In contrast to the results obtained from the superior sagittal sinus, no difference between LAEV and LACCA could be demonstrated after 15 minutes of reperfusion.

FIGURE 3. Bar graph of mean±SEM differences (Δ %) in lactate concentrations of external jugular vein and common carotid artery (cross-hatched bars) and of superior sagittal sinus and common carotid artery (filled bars) for three rats. Differences from two sources dissociate significantly between 15 and 180 minutes after 10 minutes of forebrain ischemia but show tendency to harmonize again at 180 minutes. *p<0.05 different from cross-hatched bar by Kruskal-Wallis analysis of variance followed by Mann-Whitney U test.

Discussion

Our data demonstrate an increased output of LA from brain tissue to venous blood for up to 3 hours after forebrain ischemia. This pathologic LA response could be detected in the superior sagittal sinus but not in the external jugular vein. The level of significance between the $\Delta_{LAEJV}$—$\Delta_{LACCA}$ and $\Delta_{LASS}$—$\Delta_{LACC}$ differences at 180 minutes (Figure 3) furthermore indicates that the cerebral output of LA is still elevated at that time, when LA concentrations in the systemic venous and arterial blood have returned to control levels.

LA has been observed to accumulate in ischemic brain tissue1-9 and to play a role in the pathophysiology of cerebral ischemia2-9 and traumatic brain injury.16-20 Conflicting reports exist about the permeability of the BBB to LA. Posner and Plum21 concluded from comparisons of arterial, cerebral venous, and cerebrospinal fluid concentrations of LA in human as well as experimental studies under a variety of pathologic conditions of the brain (not including cerebral ischemia, however) that the permeation rate of LA across the BBB would be slow and that cerebrospinal fluid would provide a better index of the concurrent brain LA levels. Similar results were obtained by others, who could not demonstrate an increased output of LA to cerebral venous blood.22,23 Oldendorf,13 on the other hand, demonstrated that the BBB was readily permeable to LA using intracarotid injections of 14C-labeled tracers, such as L-lactate, in rats. This mechanism was found to be bidirectional, saturable, stereospecific, and eventually carrier-mediated. Oldendorf13 concluded that elevated brain LA concentrations during ischemia could seriously impede the brain LA washout by carrier saturation. Nemoto and Severinghaus24 in agreement with the findings of Oldendorf, reported a mechanism of facilitated transport for LA from arterial blood to brain and assumed that this mechanism was bidirectional. Zimmer and Lang1 reported increased LA output from brain to cerebral venous blood in isolated dog brain and found a small trend in favor of facilitated transport of LA from brain to venous blood, which would be saturated early.

Our findings support the hypothesis that the BBB is readily permeable to LA. Estimation of LA concentrations in the cerebral venous blood could provide valuable insights into the metabolic state of the brain in a dynamic fashion on a long-term basis without interference from anesthetic agents or restraint in small laboratory animals. The fluctuations in LASS were not related to changes in arterial LA concentration, which normalized quickly after an initial increase upon reperfusion. The transient rise of $\Delta_{LCCA}$ is most likely attributed to a systemic production of LA during systemic hypotension.16,23-27

Of particular interest are the findings of Inao et al.,19 who assessed the dynamics of brain tissue LA compared with cerebrospinal fluid LA and the arteriovenous difference in LA concentrations following
cerebral fluid-percussion injury in cats. That study emphasized the need for caution in interpreting cerebrospinal fluid LA concentrations and demonstrated a better correlation between brain tissue LA and the arteriovenous LA difference, especially in the presence of systemic metabolic changes.

Conflicting reports exist concerning the time course of the pathologic LA increase in brain tissue after forebrain ischemia caused by carotid clamping and systemic hypotension. Using nuclear magnetic resonance spectroscopy (NMRS), Peeling et al reported pathologically elevated LA concentrations in extracts of variable brain regions for as long as 7 days after 10 minutes of forebrain ischemia associated with systemic hypotension in rats. Using enzymatic fluorometry in the same model of global ischemia, Biros and Dimlich showed that elevated LA concentrations in brain tissue extracts returned to near normal 90 minutes after 30 minutes of forebrain ischemia. This was in good agreement with findings of Pulsinelli and Duffy, who demonstrated significantly elevated brain tissue LA concentrations for 1 hour after 30 minutes of forebrain ischemia. This apparent inverse relation between the duration of ischemia and the duration of elevated LA levels after 10 and 30 minutes of forebrain ischemia is surprising since a recent study by Allen et al., using NMRS measurements of LA in tissue extracts, indicated that the time course and magnitude of pathologic cerebral LA levels were directly proportional to the duration of forebrain ischemia.

Our data indicate that cerebral LA output increased for at least 3 hours after 10 minutes of forebrain ischemia. Assessment of the cerebral venous-arterial difference in LA concentration provides insights into the impaired metabolic state of the postischemic brain rather than into the actual concentrations of LA in brain tissue. Therefore, our technique would allow repeated estimation of the metabolic state of the brain under almost on-line conditions. Since neither anesthesia nor restraint is needed, the method could be combined with models of conscious ischemia, which would minimize the side effects generated by pharmacologic agents and stress.

References


Key Words • cerebral ischemia • cerebral veins • lactate • rats
Increased cerebral lactate output to cerebral venous blood after forebrain ischemia in rats.
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