Lactate Uptake and Metabolism by Brain During Hyperlactatemia and Hypoglycemia

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Abstract

Entry into CSF and consumption by brain of blood-borne lactate (La) was quantified in pentobarbital-anesthetized, normocapnic dogs loaded and infused with NaLa and HLa to hold constant, in arterial blood, both the La concentration at about 8 mM (normal = 1 mM) and the pH at 7.4. In four dogs studied hourly over six hours, the arteriosagittal sinus blood concentration difference (ΔA-V La) was 0.41 ± 0.14 (SE) mM (P < 0.05) and was time independent. CSF La rose slowly over four hours to about 0.6 of blood La while cisternal CSF pH remained nearly constant. Four acetate loaded controls showed no changes of ΔA-V La, CSF La, CSF, or arterial pH. Brain uptake of La was quantitated in eight dogs during insulin-induced hypoglycemia, to minimize possible competition by glucose. Cerebral blood flow (CBF) and ΔA-V for La, glucose, and O₂ were determined at 30-minute intervals. CBF and cerebral metabolic rate of O₂ (CMRO₂) both fell about 17% during two hours of hypoglycemia and returned to control with La loading although blood glucose continued to fall to 1.5 mM. In the two hour La loaded period ΔA-V La was 0.27 ± 0.10 mM (n = 32) and CSF La rose to 0.7 of arterial La without altering CSF pH. CMRO₂ averaged 1.61 ± 0.14 μ mol/(min-gm brain), of which CMR glucose (X6 to give O₂ equivalents) provided 75% or 1.18 ±0.13 μ O₂ eq/(min-gm). CMR La X 3 was 28% of CMRO₂ or 0.45 ± 0.15 μ O₂ eq/(min-gm). The results suggest that blood-borne La can stoichiometrically replace about one-fourth of the glucose used as brain substrate during hypoglycemia, and probably during normoglycemia. Uptake may be limited by saturation of carriers facilitating passage of La across the blood-brain barrier and into brain cells.

Introduction

The questions addressed by this investigation are whether the brain can continuously metabolize lactate (La) or lactic acid delivered to it in the blood, and what fraction of its substrate need can be met by La under optimum conditions, i.e., high blood La level, low blood sugar level and Pao₂ > 100 mm Hg. The problem hinges on the permeability of the "blood-brain barrier" to lactate or lactic acid. The barrier here consists not only of the capillary endothelium, but the walls of the cells in which La is to be metabolized. Several recent experiments have shown that perhaps as much as 15% of the blood-borne ¹⁴C labeled L-lactate crosses from blood to brain in one passage through the brain. This, however, does not establish either penetration into brain cells or net uptake and metabolism. Several reports cast doubt on the quantitative transport of La into brain by showing that neither brain nor CSF La levels were increased 15 to 40 minutes after intravenous injection of large amounts of sodium lactate. Furthermore, Crone and Sørensen were unable to detect brain uptake of labeled La during passage of blood through the brain. Posner and Plum, observing small, sometimes "negligible" A-V La differences across the brain when CSF La was greatly elevated in both dogs and patients, concluded that La crosses the blood-brain barrier slowly. These reports conflict both with the labeled La uptake and with other reports showing significant amounts of La moving either out of the brain during hypoxia or into the brain during hypoglycemia or lactacidemia. The quantitative aspects of such La movements are important not only to an understanding of brain aerobic and anaerobic pathways, but also to characterization of the role of brain lactic acid metabolism in its own acid base balance, particularly as this relates to the regulation of cerebral microcirculation and the chemical regulation of respiration. If blood-brain barrier La transport is via saturable carriers, they may be rate limiting in either the excretion of anaerobically produced La or the uptake and aerobic metabolism of blood-borne...
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La. Cohen et al. estimated the brain La excretion in hypoxic man (Pao<sub>2</sub> = 35 torr) to be about 20% of glucose consumption, expressing both in O₂ equivalents. If this amount proved to be a transport-limited maximum, it might be anticipated that lactic acid would accumulate within the brain and contribute to vasodilatation and hyperventilation.

Methods

Mongrel dogs (15 to 25 kg body weight) were anesthetized with sodium pentobarbital I.V. in a dose of 30 mg per kilogram plus 5 mg per kilogram-hour, paralyzed with gallamine (102 mg per kilogram-hour), and ventilated via tracheostomy with 40% to 60% O₂ to a constant end-tidal P<sub>CO₂</sub> of 40 torr, monitored by an infrared CO₂ analyzer. Polyethylene catheters were placed into a femoral artery (for sampling and blood pressure recording), and both femoral veins for I.V. infusions. Thermostated heaters were used to keep rectal temperature at 37.5 ± 1.0°C.

Cerebral venous blood was sampled by a polyethylene catheter cemented (Eastman Kodak 910) into the superior sagittal sinus through a burr hole with the catheter tip about 2 to 3 cm rostral to the torcula. CSF samples were drawn from a polyethylene catheter similarly cemented into the cisterna magna after exposure of the atlanto-occipital membrane. The dogs were in the supine position.

Blood lactate concentration was elevated by infusing a priming dose of 200 mmole of 1 M NaLA (pH adjusted to 7.3) over a five to ten-minute period, followed by infusion at 200 mmole per hour of either 1 M NaLA or 0.6 M HLA as needed to maintain arterial pH at 7.4. Four control dogs were given 1 M sodium acetate (pH adjusted to 7.3) and 0.6 M acetic acid instead of NaLA and HLA.

Arterial and cerebral venous blood samples were drawn simultaneously. Oxygen content of the blood samples was determined by Klingenmaier’s method in which P<sub>o₂</sub>, P<sub>CO₂</sub>, and pH were determined at 37°C in appropriate electrodes.

Blood and CSF lactate concentrations were determined enzymatically by the method of Bergmeyer using 1.0 ml of nonheparinized blood or 0.5 ml CSF transferred immediately into two volumes of ice-cold 6% perchloric acid, determining dilution by weight. Glucose concentration was measured after 50-fold anaerobic dilution with oxygen-free carbon monoxide-saturated distilled water. P<sub>o₂</sub>, P<sub>CO₂</sub>, and pH were determined at 37°C in appropriate electrodes.

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CBF was determined by the washout of 1<sup>32</sup>Xe from the brain following injection, through a nonocclusive catheter cemented into the left common carotid artery, of about 0.1 ml saline containing about 0.3 mCi of the isotope. Brain 1<sup>32</sup>Xe activity was determined by external scintillation detection utilizing a 1" × 1½" sodium Nal-thallium activated crystal with an integral photomultiplier unit (Harshaw), and a flat field collimator with a one-half energy angle of 30° (skull-crystal distance of approximately 8 cm). The left temporal and parietal skin and musculature were reflected out of the counting field. The slope of the linear regression of In count rate versus time, d(InXe)/dt, was computed during the first minute of washout, beginning six seconds after peak activity was detected.

\[ \text{CBF} = \lambda (\text{d(InXe))/dt, ml/(gm-min)}, \]

where \( \lambda = 1.08, \)
The whole brain to blood partition coefficient for Xenon.

Results

Cerebral arteriovenous lactate concentration differences (ΔA-V La) were determined in four dogs over a one-hour control period and a six-hour lactate loaded period, and in four control dogs loaded with acetate over the same period. At the end of the ten-minute injection of 200 mM of NaLa, arterial La was 16 mM, and ΔA-V La was 2.6 ± 1.4 mM. Figure 1 illustrates the rise of CSF La progressively over six hours to about 6 mM with t½ (time to one-half equilibration with arterial La) of 3.5 hours. The mean ΔA-V La values at each sample period are given in Table 1. ΔA-V La (excluding the ten-minute sample) averaged 0.407 ± 0.145 (n 24), a difference significant at P < 0.05, with no significant time dependent trend. The controls showed no significant ΔA-V La (mean = −0.05 ± 0.02 mM), and no changes in blood, CSF lactate, or pH. La loaded dogs showed a small fall in CSF pH from the fourth to sixth hours, possibly a result of imperfect control of arterial pH by balancing of the infused NaLa and HLA.

Cerebral consumption of glucose, oxygen and lactate was determined in eight animals over a 4.5-hour period consisting of a one-hour control, a 1.5-hour induction of hypoglycemia by I.V. injection of 800 to 1,600 U of bovine regular insulin, and a two-hour La loaded period. Samples were drawn at 30-.
Blood glucose fell from about 4.5 mM to 1 to 2 mM as shown in figure 2.

La loading held arterial La at about 7 to 8 mM, and CSF La rose over the two-hour loaded period to about 5 mM, with time of about two hours to reach half the arterial level.

During the last hour of hypoglycemia, CBF and cerebral metabolic rate of O₂ (CMRO₂) each averaged 17% (P < 0.05) below the mean of the last hour of the control period. Table 2 presents the mean of measured CBF, ΔA-V for La and glucose and the calculated CMR for La, O₂, and glucose for the three periods. Excluding the sample ten minutes after beginning the loading in La loaded dogs, the mean ΔA-V La was 0.27 ± 0.10, which was not significantly different from the ΔA-V La in the La loaded normoglycemic dogs studied over six hours. La loading restored CBF and CMRO₂ to control levels, of 48 ml per 100 gm·min and 1.6 μmol per gm·min, respectively, while mean CMR glucose was 1.18 ± 0.13 μO₂eq per gm·min, and CMR La was 0.45 ± 0.15 μO₂eq per gm·min (data having been multiplied by six and three, respectively, to obtain O₂ equivalent quantities). No significant changes of arterial or CSF pH, PₐCO₂, PₐO₂, body temperature, or mean arterial blood pressure were observed. The CMR glucose was not significantly different from CMRO₂ during the control and hypoglycemic periods, and no significant uptake or elimination of La occurred during these periods (table 2). The CMR glucose and CMR La represented 75% and 28% of CMRO₂, respectively, during the lactate loaded period. The mean influx of La was 0.13 μmol of La, or 0.45 μO₂eq per gm·min, although the sample taken at ten minutes after beginning the loading showed an influx of about twice this amount at a time when arterial La concentration averaged 14 mM.

**Discussion**

Brain consumption of blood-borne La averaged 4% to 5% of the arterial lactate entering the brain over six hours without hypoglycemia, and over two hours with hypoglycemia. Hypoglycemia failed to increase this fractional extraction. The La uptake induced by La loading during hypoglycemia was sufficient to restore O₂ consumption and CBF to control levels, and the stoichiometric sum of glucose and lactate uptake was not significantly different from the O₂ consumed. There appeared to be no progressive decrease of ΔA-V La in either group of animals to suggest that the ΔA-V La was due to saturation of brain tissue with La. The observed La uptake would raise brain lactate concentration 8 mM per hour if none were consumed. If the whole brain La concentration rose at the rate seen in CSF, about 1 to 2 mM per hour, 12% to 25% of observed CMR La could have been stored rather than metabolized. The data are not sufficiently precise to detect such a discrepancy.

In both groups of animals, the ΔA-V La was significantly higher at the end of the initial ten minute loading period when arterial La was 14 to 16 mM. While this increased initial uptake may be mostly stored rather than metabolized, the data nevertheless indicate that the flux of lactate is concentration dependent. This is more consistent with transmembrane diffusion than with a saturable carrier. A major portion of this influx could be accounted for by saturation
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TABLE 2

Brain-Blood Flow, Oxygen, Glucose and Lactate Metabolism During Insulin-Induced Hypoglycemia* I.V. Lactate Infusion† in Eight Dogs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Hypoglycemia</th>
<th>La loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>La (mM)</td>
<td>mean 0.45 ± 2.9</td>
<td>-0.04 ± 0.02</td>
<td>-0.04 ± 0.02</td>
</tr>
<tr>
<td>A-V La (mM)</td>
<td>0.03 ± 0.02</td>
<td>0.38 ± 0.07</td>
<td>0.38 ± 0.06</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>0.73 ± 0.07</td>
<td>0.65 ± 0.09</td>
<td>0.65 ± 0.06</td>
</tr>
<tr>
<td>CMR (µg/gmin)</td>
<td>2.76 ± 0.46</td>
<td>1.49 ± 0.21</td>
<td>1.49 ± 0.23</td>
</tr>
<tr>
<td>O₂ (mM)</td>
<td>1.98 ± 0.33</td>
<td>1.52 ± 0.14</td>
<td>1.52 ± 0.05</td>
</tr>
</tbody>
</table>

*800 to 1,600 U bovine regular insulin I.V.
†Bolus of 200 mmole 1 M NaLa (pH = 7.3) followed by continuous infusion of 1 M NaLA or 0.6 M HLa at a rate of 200 mmole per hour.
‡Not included in mean.

of brain ECF or more permeable, but less metabolically active, cells such as glia. This conjecture is supported by the data at the ten-minute point: glucose and O₂ consumption were equal (within 10%) at 1.1 µO₂eq per gmin and were 23% below control. At the same time, the La uptake by the brain was 0.70 µO₂eq per gmin, the excess uptake probably not yet having penetrated into the highly metabolically active brain cells.

La entering CSF may do so either through choroid plexus or by diffusion from brain ECF. Both barriers are sufficiently impermeable that the CSF La rose too slowly to be observed before one hour, as had been noted by both Alexander et al.1 and Leusen et al.2 Klein and Olsen1 found no elevation of brain La 40 minutes after loading blood with La. This does not imply low permeability unless it is established that entering La is not metabolized. Brain ECF is in the diffusion pathway between blood and brain cells, and may be presumed to have an La concentration between arterial and brain cell La. The steady uptake and metabolism of La by brain from one to six hours suggests that brain cell level is not gradually rising, and probably means that La entering the cells is metabolized without a progressive rise in intracellular La. The inference is that brain ECF La also had stabilized within one hour of La loading, and the gradual rise of CSF La was due to the slow exchange of the pool of ventricular or cisternal fluid with both brain ECF and newly formed CSF. The exchange of La between CSF and brain was studied by Prockop17 in a ventricular-cisternal perfusion system. He demonstrated a relatively slow efflux from CSF by comparison with a non-metabolized glucose analog, 3-O-methyl D-glucose which utilizes the glucose facilitated transfer, but rapid in comparison with insulin. He concluded that La removal was not via a facilitated transport, although he did not test other weak acids.

The rate of penetration of weak acids and other materials into CSF from blood has been investigated by Rall et al.18 In dogs, sulfadiazine, a weak acid, exhibits a half time of equilibration with CSF of about one hour, with a final concentration of 0.8 of plasma concentration due to the somewhat lower pH in CSF. A similar ratio would be expected for La. In the normoglycemic dogs our final ratio was 0.6 for the last three hours, but the blood level was rising slightly over this time, preventing estimation of the steady state CSF/arterial La concentration ratio. The half times estimated for our data relate to the CSF to arterial difference rather than to a final CSF plateau and hence are longer than would apply if we had prolonged the experiment sufficiently to determine the final CSF/arterial La ratio. The more rapid rise of CSF La in the hypoglycemic dogs may be a result of increased frequency and volume of sampling. It is notable that the rising CSF La level did not reduce CSF pH, suggesting that the entering species may not have been HLa, whereas the species undergoing metabolism in cells is HLa. CSF may be misleading as an index of brain cellular or ECF La concentration, although, as pointed out by Posner and Plum,4 it is a far better index than cerebral venous blood when brain and CSF lactate are elevated above blood levels.

Ashford and Holmes9 determined the oxidation
of La by chopped frog brain, and concluded that it increased oxygen uptake, and appeared not to spare glucose. McGinty\textsuperscript{10} had shown in 1929 that normoxic brain takes up lactate from venous blood, while ischemic, or hypoxic, or cyanide-exposed brain excretes lactic acid. In 1941, Wortis et al.\textsuperscript{12} attempted to treat hypoglycemic shock in man by intravenous infusion of NaLa. They reported that it was ineffective in relieving the symptoms, but they documented uptake by measuring arterial and internal jugular La levels. Sacks\textsuperscript{20} also attempted to measure brain uptake of La in man using radioactive labeled compound, but did not rule out the possible pathway in which the label was resynthesized to glucose in the liver before entering the brain. And Cohen et al.\textsuperscript{9} using volunteers, obtained A-V La differences suggesting that, during hypoxia (Pa\textsubscript{0,2} = 35 torr) for 15 minutes, brain extraction of lactate rose from 4\% to about 19\% of the glucose consumed. None of these studies included serial A-V concentration differences, over several hours of steady state, to preclude the possibility that the observed A-V differences were related to changes in brain La stores rather than in brain La metabolism. Plum and Posner\textsuperscript{31} have obtained such steady state measurements in dogs hyperventilated to Pa\textsubscript{0,2} = 10 torr. Six normoxic dogs studied hourly over six hours showed a rather constant mean \(\Delta A-V\) La of \(-0.57\) mM, with arterial and CSF La values of 3.5 and 7.6 mM (CSF mean of last four hours). If we assume that cerebral blood flow was half of normal (the lower limit produced by hyperventilation) the lactate production may be estimated as: \(-0.57\) mM \(\times 0.46/2\) (CBF/gm) \(\times 3 = 0.4\) \(\mu \text{g} \text{ eq} \text{ per g} \text{ min}\), a value comparable to the uptake of La in our hypoglycemic animals. Plum and Posner\textsuperscript{31} found a similar mean \(\Delta A-V\) La, \(-0.51\) mM, in eight similarly hyperventilated but hypoxic dogs over six hours, with arterial and CSF values averaging 4.2 and 11.0 mM, respectively, and with indications that hypoxia increased blood flow (smaller \(\Delta A-V\) CO\textsubscript{2}, O\textsubscript{2} and HCO\textsubscript{3}) such that the La production may have been even greater. However, without actual determinations of flow, they were unable to quantify brain lactate excretion, and they did not determine \(\Delta A-V\) for glucose or oxygen content.

Oldendorf\textsuperscript{P} has shown that the blood-barrier is about three times more permeable to exogenous L-La than to D-La, and we\textsuperscript{e} obtained a similar ratio in rats. Daniel et al.\textsuperscript{1} also used labeled La to show that influx into the brains of rats was about half that of glucose. We have found, as has Oldendorf, that the threefold facilitation of L-La over D-La was blocked by elevation of blood La. The remaining loss of lactate from blood passing the brain in Oldendorf's study was 5\% of the arterial La comparable to our two present steady state groups. This also suggests that the La concentration in brain cells is not elevated sufficiently to decrease the net influx across carrier-saturated membranes. The evidence suggests that at arterial La concentrations of 8 mM, uptake and metabolism of La are limited to about 25\% of total substrate requirement, independent of blood glucose concentration. This suggests but does not prove that transport by the saturated carrier is the limiting factor in determining the fraction of brain metabolism that can be supplied by La. Another possibility which cannot be excluded, by these results, is that brain can metabolize La only to the extent of about 25\% of its needs, whether intracellular La concentration is high or low.

A stereospecific saturable facilitated transport for L-La in normal brain function may affect brain acid base balance by permitting excretion of small amounts of La in steady states of tissue hypoxia. As lactate production by hypoxic brain rises past the saturation level of transport, the La gradient from brain to blood will increase out of proportion, resulting in steeply rising brain intracellular and perhaps ECF concentrations, which produce vasodilation and respiratory stimulation. Such considerations, in fact, motivated McGinty's study\textsuperscript{10} long before the location and nature of the chemosensitive respiratory neurons or the apparent direct relationship of pH to arteriolar resistance were established.

The nature of La transport beyond the saturation of the carrier, such as that observed at the end of the ten-minute loading period, we presume may be due to simple diffusional influx of neutral lactic acid molecules which comprise about 1 in 2,700 bloodborne La and may apply only to the endothelial or glial cells rather than to all brain cells.

Loading was done with ordinary lactic acid neutralized with NaOH. The supply used was determined to be about 80\% L-La and 20\% D-La, assuming the enzymatic analysis detects only L-La. The metabolism of D-La in brain, while occurring, is slower than that of L-La. The present results therefore fail to measure the total La metabolism by brain.

The accuracy of analysis of CMR La is primarily limited by determination of the difference between arterial and venous La concentrations, which averaged about 3\% to 5\% of the arterial level, a problem which is intensified by loading to high blood levels. The mean \(\Delta A-V\) La of eight animals was not significantly different from zero at \(P = 0.05\) at any one time of sampling except at the end of the ten-minute loading period. The mean \(\Delta A-V\) La of the entire loaded period was significantly different from zero in both normoglycemia and hypoglycemia.

References

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