Stereospecific Permeability of Rat Blood-Brain Barrier to Lactic Acid

BY EDWIN M. NEMOTO, PH.D., AND JOHN W. SEVERINGHAUS, M.D.

Abstract:
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Blood and whole brain $^{14}$C and $^{32}$P activities were determined in hepatectomized rats one, two, five and ten minutes after intravenous (I.V.) injection of $^{14}$C-labeled L-lactate or D-lactate and $^{32}$P-labeled rat red blood cells. Whole brain homogenate $^{14}$C was corrected for blood $^{14}$C and chemically partitioned into $^{14}$C-lactate, $^{14}$CO$_2$ and other $^{14}$C compounds. In controls, lactate was replaced with $^{14}$C-D-glucose and $^{125}$I-antipyrine. At one minute postinjection, whole brain $^{14}$C expressed as percent of total injected $^{14}$C activity and as percent of the antipyrine value were: antipyrine 1.78% (100%); D-glucose 1.45% (81%); L-lactate 0.36% (20%); and D-lactate 0.13% (7%). One minute after L-lactate injection, brain $^{14}$C was 74% lactate, 5% CO$_2$ and 21% other compounds. Preloading rats with cold racemic Na-lactate reduced L-lactate uptake to 0.14% of the injectate (8% of antipyrine), and reduced D-lactate uptake to 0.09% (= 5% of antipyrine). At two, five and ten minutes, brain contained more $^{14}$C with larger fractions metabolized to CO$_2$ and other compounds from both L-lactate and D-lactate. The blood-brain barrier appears to contain a saturable lactate carrier exhibiting threefold L-stereospecificity to D-stereospecificity, but resulting in far less net transport than the comparable glucose carrier. Lactate transport may be limited by the scarcity of neutral lactic acid at normal blood pH.

Introduction
Cerebrospinal fluid (CSF) pH plays an important role in the regulation of respiration$^1$ and cerebral blood flow.$^2$ During hypoxia,$^3$ ischemia$^4$ and hypocapnic alkalosis$^5$ CSF pH changes are moderated by alterations in lactic acid (LA) concentration, which is a function of: (1) brain LA production and metabolism, (2) blood-brain and blood-CSF LA permeability, and (3) CSF pH itself. Factors contributing to the importance of LA in determining CSF pH are: (1) high metabolic rate of brain$^6$ with low oxygen stores$^7$ and, therefore, higher sensitivity to oxygen lack, and (2) low permeability of the blood-CSF and blood-brain barrier (BBB) to ions$^8$ (at a pH of 7.4 only one out of 2,700 LA molecules exists in the nonionized form). In this study we have attempted to determine LA permeability and mechanism of transport across the BBB.

Several studies have demonstrated bidirectional net transport of LA across the BBB. McGinty$^9$ and Wortis$^8$ reported net uptake or excretion of LA by brain. The method of LA assay in these experiments were of questionable accuracy and specificity.$^{10, 11}$ O’Neal and Koenpe$^{12}$ and Sacks$^{13}$ showed LA exchange between blood and brain in $^{14}$C-labeled LA studies. Sacks also reported more rapid brain metabolism of L-LA than of D-LA. These steady state tracer studies were complicated by the possibility that labeled LA could be resynthesized to labeled glucose and metabolized by the brain. During hypoxia, Cohen et al.$^{14}$ obtained evidence suggesting that 25% of the glucose consumed by the brain was converted to LA. Sørensen et al.$^{15, 16}$ confirmed these findings with hypoxia of similar magnitude at high altitude but CSF LA was not markedly elevated,$^1$ suggesting rapid excretion of LA by brain.

Some recent studies have refuted the studies demonstrating significant permeability of LA across the BBB. Crone and Sørensen,$^{17}$ using double indicator cerebral venous dilution curves, were unable to detect measurable exchange of labeled LA across the BBB. Alexander$^{18}$ and Leusen$^{19}$ found brain and CSF LA unchanged after 15 to 30 minutes of I.V. infusion of NaLA.

We have attempted to quantify BBB permeability of LA compared to that for glucose and antipyrine and to test its stereospecificity and saturability.

Methods
The trachea, right jugular vein and left common carotid artery were cannulated in pentobarbital-anesthetized (60 mg per kilogram intraperitoneally) female Sprague-Dawley rats (200 to 400 gm body weight). The hepatic artery and hepatic portal veins were ligated with a suture (at the hepatic trigone) for ligation ten seconds before the I.V. injection of...
the isotopic mixture. Blood was drawn from the carotid artery immediately before the injection of the isotope for enzymatic determination of arterial LA, and for \( p_{oa} \) and \( p_{co} \), and pH determinations.

The following labeled compounds were used: sodium D-LA\(^{14}C\), s.a., 9.1 mCi/m mole; sodium L-LA\(^{14}C\), s.a., 56 mCi/m mole; D-glucose\(^{14}C\), s.a., 3.0 mCi/m mole; and antipyrine\(^{3}H\) (Amersham/Searle). Ascending paper chromatography of the \( {^{14}C}\)-L-LA and D-LA (n-butanol:water:acetic acid, 2:5:3) and D-glucose (n-butanol:ethanol:water, 52:33:13) showed essentially 100% of the \( ^{14}C \) activity as the labeled molecule. Rat erythrocytes were labeled by the incubation of 10 cc of blood, with approximately 60 \( \mu \)Ci of carrier-free \( H_{3}^{3}PO_{4} \). After a two-hour incubation period, the cells were washed with saline four times and suspended in saline at a hematocrit of approximately 40%. The injected solution contained about 2 \( \mu \)Ci of the test substance and about 6 \( \mu \)Ci of \( ^{32}P \)-tagged cells in a total volume of approximately 1.5 ml. The \( ^{32}P \)-labeled cells permitted correction of whole brain homogenate \( ^{14}C \) activity for trapped blood \(^{14}C \) activity. The rats were decapitated at one, two, five and ten minutes postinjection.

Immediately following decapitation, blood was collected from the neck, and the entire brain was rapidly removed and frozen in liquid \( N_{2} \) (see DISCUSSION for LA metabolism prior to freezing). One-half of each brain and blood sample were homogenized in 0.1N KOH and the other half in 0.1N HCl (tissue:KOH or HCl ratios of 1:4 for brain and 1:1 for blood), the difference between them indicating the activity of \( ^{14}CO_{2} \). HCl homogenized samples were vacuum (20 cm Hg) extracted with stirring for ten minutes. Brain (0.2 ml) and blood (0.2 ml) homogenates were then solubilized at room temperature with 1.2 and 0.6 ml NCS solubilizer (Nuclear-Chicago, respectively). Blood samples were bleached by the addition of 0.07 ml of benzoyl peroxide (6 gm/30 ml) in toluene. \(^{14}C\) and \(^{32}P\) activities were determined by liquid scintillation counting (Packard Tri-Carb) in a PPO/toluene (6 gm/L) mixture. Channel efficiencies were determined by means of internal standards.

Brain and blood \(^{14}C\) metabolites (i.e., glucose, pyruvate, malate, etc.) other than \(^{14}C\)-LA were determined by treatment of 0.1N HCl homogenized samples with the Barker-Summoner copper-amber precipitation technique and counted in Bray's solution. In test extractions, 99% of \(^{14}C\)-labeled D-glucose was removed, while 95% to 98% of L-LA was retained.

Regional concentrations of \(^{14}C\) (L-LA) in cortex, midbrain, cerebellum and medulla also were determined in two rats killed at one minute. Four rats were loaded with racemic NaLa (0.7 mmole per kilogram, i.v.) ten minutes before \(^{14}C\) injection to test for saturaibility of LA transport.

### Calculations

Whole brain \(^{14}C\) or \(^{15}I\) activity (G) as percent of injected activity (F) was calculated as:

\[
G = \left( C - DA/B \right) / \left( 100 \times E/F \right),
\]

where \( A \) = brain \(^{32}P\), dpm per gram; \( B \) = blood \(^{32}P\), dpm per milliliter; \( C \) = brain \(^{14}C\), dpm per gram; \( D \) = blood \(^{14}C\), dpm per milliliter; and \( E \) = brain weight, gram.

### Results

Arterial pH, \( p_{oa} \), and \( p_{co} \), were similar in both \(^{14}C\)-L-LA and \(^{14}C\)-D-LA injected rats (table 1). Ten-minute arterial LA values were significantly elevated in both groups (table 2), which probably is a consequence of the functional hepatectomy.

Whole brain \(^{14}C\) activity in 0.1N KOH homogenized samples (except for one-minute groups which were 0.1N HCl homogenized) is shown in table 2. At one, two and five minutes, significantly more \(^{14}C\)-L-LA than \(^{14}C\)-D-LA had entered the brain \((P < 0.05)\). Whole brain \(^{14}C\)-L-LA to \(^{14}C\)-D-LA ratio at one minute was 2.9.

Metabolism to \(^{14}CO_{2}\) and other \(^{14}C\) compounds was detectable for L-LA at one minute and was significantly faster for L-LA than for D-LA at two and five minutes (table 2).

The distribution of \(^{14}C\) activity in HCl homogenized brain of two rats studied at one minute was relatively uniform: cortex, 0.172; midbrain, 0.131; cerebellum, 0.150; and medulla, 0.142 (percent of total injected activity per gram tissue).

Rats preloaded with racemic NaLA ten minutes before \(^{14}C\) injection were killed at one, two, five and ten minutes postinjection. The following labeled compounds were used:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Activity (mCi/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium D-LA</td>
<td>56 mCi/mole</td>
</tr>
<tr>
<td>Sodium L-LA</td>
<td>9.1 mCi/mole</td>
</tr>
<tr>
<td>D-glucose</td>
<td>3.0 mCi/mole</td>
</tr>
</tbody>
</table>

**Table 1**

<table>
<thead>
<tr>
<th>Min postinjection</th>
<th>pH</th>
<th>( p_{ao} ) (mm Hg)</th>
<th>( p_{co} ) (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>X</td>
<td>7.331</td>
<td>44.0</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.023</td>
<td>3.6</td>
</tr>
<tr>
<td>2</td>
<td>X</td>
<td>7.364</td>
<td>39.1</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.020</td>
<td>2.0</td>
</tr>
<tr>
<td>5</td>
<td>X</td>
<td>7.360</td>
<td>40.8</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.018</td>
<td>1.0</td>
</tr>
<tr>
<td>10</td>
<td>X</td>
<td>7.343</td>
<td>41.2</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.027</td>
<td>3.3</td>
</tr>
</tbody>
</table>

*Minutes postinjection refers to time of decapitation after injection of the labeled lactate.
BRAIN BARRIER LACTATE PERMEABILITY

Table 2

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Isomer</th>
<th>n</th>
<th>Blood (LA) μmoles/ml</th>
<th>Whole brain 14C activity (% of total act. injected)</th>
<th>Partitioned 14C act. (% of whole brain act.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L</td>
<td>5</td>
<td>1.79 ± 0.24</td>
<td>0.360 ± 0.049</td>
<td>74 5 5 21 2</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>5</td>
<td>1.18 ± 0.14</td>
<td>0.126 ± 0.017*</td>
<td>88 10† 5 2† 2</td>
</tr>
<tr>
<td>1</td>
<td>L</td>
<td>4</td>
<td>2.83 ± 0.59</td>
<td>0.141 ± 0.013</td>
<td>--- --- --- ---</td>
</tr>
<tr>
<td>(lactate loaded)</td>
<td>D</td>
<td>4</td>
<td>3.29 ± 0.56</td>
<td>0.089 ± 0.014*</td>
<td>--- --- --- ---</td>
</tr>
<tr>
<td>2</td>
<td>L</td>
<td>4</td>
<td>1.42 ± 0.36</td>
<td>0.546 ± 0.144†</td>
<td>31 27 3 42 2</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>5</td>
<td>1.42 ± 0.38</td>
<td>0.192 ± 0.014†‡</td>
<td>66 13 5 21 2</td>
</tr>
<tr>
<td>5</td>
<td>L</td>
<td>4</td>
<td>1.58 ± 0.15</td>
<td>0.540 ± 0.134</td>
<td>24 27 4 49 2</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>4</td>
<td>1.60 ± 0.04</td>
<td>0.180 ± 0.042*</td>
<td>72 15 4 13 2</td>
</tr>
<tr>
<td>10</td>
<td>L</td>
<td>4</td>
<td>2.49 ± 0.17</td>
<td>0.484 ± 0.122</td>
<td>45 15 4 40 2</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>3</td>
<td>2.61 ± 0.27</td>
<td>0.277 ± 0.107</td>
<td>35 24 3 41 2</td>
</tr>
</tbody>
</table>

Column 4 is the nonlabeled blood lactate concentration, which was elevated by preloading in four animals. Column 5 is the percentage of the total injected 14C which was recovered in brain after correcting for blood 14C trapped in the brain. The CO2 fraction is the difference in homogenate radioactivity between KOH and HCl homogenation. Other compounds are those removed by copper-lime precipitation, including glucose, pyruvate, malate and other citric acid cycle intermediates.

* D < L.
† t Two minutes > one minute.

Before injection of label showed less brain uptake of label at one minute for both L-LA and D-LA (P < 0.05), even though loading increased arterial lactate levels only two to two and one-half times normal.

Two rats were given 14C-D-glucose and two given 14C antipyrine, both with 35P-labeled erythrocytes, to determine the percent of injectate found in whole brain at one minute for a carrier-mediated and a freely permeable, simple diffusion substance. Of the total injected activity, 1.78% of the antipyrine and 1.45% of the glucose were recovered in the brain. By comparison with antipyrine as 100%, at one minute the brain uptake of glucose (81%), where transport across the BBB also is carrier-mediated, and LA may be due to fewer carrier sites for LA or scarcity of the ionized species of LA at pH 7.4.

Why were three groups of investigators unable to show BBB permeability to LA? In two studies, neither brain nor CSF LA was elevated after 15 to 30 minutes of I.V. infusion of LA. The lack of increase in brain or CSF LA after LA infusion may be due to a limited influx of LA; brain metabolism of inwardly diffusing LA, or slow turnover of CSF. In a subsequent study, we found that in dogs the CSF LA can be gradually elevated after one to three hours if blood LA is raised to 5 to 10 μmoles per milliliter.

Crone and Sørensen reported no extraction of D-LA or L-LA from a single bolus of 14C-LA passing through the brain as compared to 3H-mannitol, an intravascular reference tracer. Sørensen has examined the problem involved in his experiment (personal communication) and concluded that two factors may have obscured the small LA uptake by brain, which has been reported by Oldendorf and by us. First, mannitol, which was used as their reference tracer, is slightly more permeable, at least through the choroid plexus, than inulin, and inulin, according to Crone, shows an extraction of 2.5% compared to albumin-bound Evan's blue dye. Second, LA enters erythrocytes more rapidly than mannitol, with kinetics which make estimation of plasma activity during transit difficult.

It is of interest that brain uptake of D-LA was significantly depressed by loading with racemic lactate, although not as dramatically as that of L-LA.
This would suggest that a carrier is functioning for both. Whether it is the same carrier remains to be established by studying the effect of loading with one isomer on transport of the other.

Oldendorf's method,\textsuperscript{22} in which the test and permeable indicators are injected into an artery supplying the brain, rather than intravenously, offers advantages over our technique in terms of the correction for residual label in the blood at the time of sacrifice. Unfortunately, with his technique (decapitation at 15 seconds), it is difficult to obtain a relevant blood sample to demonstrate the absence of label from intracerebral blood. However, the two techniques have yielded comparable results.

A large fraction of the \textsuperscript{14}C-LA which entered the brain was converted to other compounds and CO\textsubscript{2} in each group of rats except those studied at one minute. We presume this to be aerobic metabolism, terminated by anoxia shortly after decapitation. Insertion of a thermistor probe in whole rat brain followed by complete immersion in liquid N\textsubscript{2} showed that the brain completely froze in about 20 seconds. During the 20 to 50 seconds between decapitation and freezing, brain glycolysis generates LA with little further modification of the chemical nature of the \textsuperscript{14}C-labeled compounds caught in transit from LA to CO\textsubscript{2}. Therefore, freezing probably is not essential in these experiments.

While the LA loading experiments showed significant suppression of \textsuperscript{14}C-LA uptake, they were incomplete in that the entire saturation curve remains to be defined. Such information might be relevant to the ability of the brain to excrete LA during and after hypoxic episodes, the effectiveness of LA as a moderator of CSF pH, and therefore of respiration and CBF during hypoxia and hypocapnic alkalosis.

References

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Stroke. 1974;5:81-84
doi: 10.1161/01.STR.5.1.81

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