Carrier-mediated blood-brain barrier transport of short-chain monocarboxylic organic acids

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The permeability of the blood-brain barrier (BBB) to 15 14C-labeled organic acids of metabolic interest was studied by injecting the test acids rapidly into the common carotid artery and decapitating the rat 15 sec later. Brain uptake during the first brain circulatory passage was measured relative to tritiated water injected simultaneously as a highly diffusible internal standard. Competitive inhibition of uptake of the radiolabeled test substance was measured by including unlabeled competitive substances in the injected solution. Uptake of straight-chain saturated monocarboxylic acids increased with chain length and was virtually complete at lengths greater than that of hexanoate. Smaller acids exhibited uptakes which were saturable and, in the case of lactate, stereospecific. No measurable uptake of di- or tricarboxylic acids was observed. Cross-inhibition was demonstrated between pyruvate and acetate, propionate, L-lactate, and butyrate but was not shown with octanoate and decanoate. Cross-inhibition was not demonstrated between pyruvate and L-phenylalanine, L-arginine, or D-glucose, indicating that the monocarboxylic acid carrier system demonstrated here is independent of blood-brain barrier amino acid and glucose transport systems.

THE BLOOD-BRAIN BARRIER (BBB) greatly retards the penetration of most lipid-insoluble solutes from plasma into brain. Certain metabolic substrates, such as glucose and some amino acids, have an insignificant lipid solubility yet penetrate the BBB readily by means of specific carrier transport systems. One carrier for glucose and two for amino acids can be readily identified as independent, saturable, stereospecific transport systems (7, 10). It is the purpose of this report to describe studies of the rate of BBB penetration of a number of small organic acids indicating that there is an accelerated rate of penetration of small, monocarboxylic acids by means of a saturable, stereospecific transport mechanism. This organic acid transport is evidently independent of glucose and amino acid transport, since cross-inhibition could not be demonstrated.

METHOD

The method employed has been described in detail (6, 7) and is only briefly summarized here.

Wistar rats of either sex, 230-350 g, were anesthetized with intraperitoneal pentobarbital. The right common carotid artery was surgically exposed, cannulated with a 0.38-mm od needle, and 0.15-0.2 ml of buffered Ringer solution (McGaw Laboratories, Glendale, Calif.) was injected during approximately 0.2 sec.

The injected Ringer solution contained 10 mm of HEPES buffer (Calbiochem, La Jolla, Calif.), approximately 0.25 μc 14C-labeled organic acid, and approximately 1 μc water-14H. In some of the studies known concentrations of unlabeled solutes were also included to study competition for saturable transport systems. The pH was adjusted to the pK of the buffer at pH 7.55 prior to injection.

Fifteen seconds after carotid injection, the rat was decapitated and the entire brain rostral to midbrain was subjected to routine liquid scintillation counting for 3H and 14C. Some of the injected solution was similarly counted.

The amount of 14C in brain was calculated as a percentage of 3H present, the 3H water serving as a highly diffusible internal standard of reference. This percentage was taken as an index of brain uptake which is some nonlinear but monotonic function of BBB permeability. The uptake index approximates the percentage of the labeled species that is lost to brain during a single capillary passage. Some representative uptakes are: approximately 100 % for ethanol and nicotine, 2-55 % for amino acids, and 32 % for D-glucose (7, 8). Nonpenetrating substances such as mannitol or mulin have an uptake of 1.8-2 %, and they provide the background level for the method. This background has not been subtracted in the results presented here.

Low injected concentrations. The 14C-labeled acids listed in Table 1 were studied at the low injected concentrations shown. The injected concentrations were those present in radiolabeled material obtained from the radiochemical supplier (New England Nuclear Corp., Billerica, Mass., or Amersham/Scarle, Arlington Heights, Chicago, Ill.).

Elevated injected concentrations. In addition to the low injected concentrations shown in Table 1, unlabeled substances were added to the injected solution to demonstrate self-inhibition and cross-inhibition for several of the acids and lack of cross-inhibition between pyruvate and glucose, phenylalanine, or arginine.

The self-inhibiting effects of the addition to the injected solution of unlabeled acid on the uptake of the same 14C-labeled acid were studied using acetate, propionate, pyruvate, L-lactate, and butyrate as test substances. The maximum concentration of unlabeled acid added was 10 mm except for L-lactate where it was carried out to 15 mm.

The cross-inhibiting effects of the addition to the injected
BBB TRANSPORT OF SHORT-CHAIN ACIDS

**Table 1. Brain uptake of **14C** organic acids**

<table>
<thead>
<tr>
<th>Organic Acid</th>
<th>Injected Conc. mM</th>
<th>Brain Uptake, % of H2O*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formic</td>
<td>0.02</td>
<td>9 \pm 2</td>
</tr>
<tr>
<td>Acetic</td>
<td>0.020</td>
<td>14.0 \pm 1.9</td>
</tr>
<tr>
<td>Propionic</td>
<td>0.009</td>
<td>31 \pm 3.2</td>
</tr>
<tr>
<td>Butyric</td>
<td>0.013</td>
<td>46 \pm 0.3</td>
</tr>
<tr>
<td>Hexanoic</td>
<td>0.010</td>
<td>70 \pm 0.8</td>
</tr>
<tr>
<td>Octanoic</td>
<td>0.043</td>
<td>94 \pm 0.6</td>
</tr>
<tr>
<td>Decanoic</td>
<td>0.022</td>
<td>86 \pm 12</td>
</tr>
<tr>
<td>Pyruvic</td>
<td>0.023</td>
<td>42 \pm 2.1</td>
</tr>
<tr>
<td>L-Lactic</td>
<td>0.011</td>
<td>16.2 \pm 1.9</td>
</tr>
<tr>
<td>Lactate</td>
<td>0.011</td>
<td>5.1 \pm 0.6</td>
</tr>
<tr>
<td>Succinic</td>
<td>0.061</td>
<td>1.9 \pm 0.4</td>
</tr>
<tr>
<td>Fumaric</td>
<td>0.104</td>
<td>1.6 \pm 3.3</td>
</tr>
<tr>
<td>Isobutyric</td>
<td>0.083</td>
<td>26 \pm 3.3</td>
</tr>
<tr>
<td>Citric</td>
<td>0.057</td>
<td>32 \pm 3</td>
</tr>
<tr>
<td>p-Aminohippuric</td>
<td>0.053</td>
<td>37 \pm 1.1</td>
</tr>
</tbody>
</table>

* For each value *n* = 3 except that for L-lactic acid, *n* = 12; for p-aminohippuric acid, *n* = 6, and for pyruvic acid, *n* = 4.

solution of unlabeled pyruvate on the uptake of **14C**-labeled acetate, propionate, butyrate, L-lactate, octanoate, and decanoate were also studied.

To establish independence (no cross-inhibiting effects) of pyruvate BBB transport from glucose and amino acid transport, 10 mM pyruvate were added to the injection of L-phenylalanine-**14C** and L-arginine-**14C** and 20 mM were added to the injection of D-glucose-**14C**.

**Results**

**Low injected concentrations.** These are shown in Table 1. All of the monocarboxylic acids other than formate have brain uptakes significantly above background. The straight-chain saturated monocarboxylic acids show uptakes increasing with chain length.

Among the three-carbon monocarboxylic acids, pyruvate has a significantly (*P* < .001) greater uptake than propionate. The hydroxylation of the center carbon in L-lactate results in a reduction of uptake, but the uptake remains well above background value.

The uptakes of L- and D-lactate are significantly (*P* < .001) different with a greater uptake of common L-lactate than the uncommon D-enantiomorph.

No measurable uptake of the di- or tricarboxylic acids was found nor was there a measurable uptake of p-amino hippuric acid (PAH). Indeed, the uptake of PAH (*n* = 6) was consistently lower than the background of the method.

**Elevated injected concentrations.** Self-inhibition of acetate, propionate, pyruvate, lactate, and butyrate is shown in Table 2. Cross-inhibition between unlabeled pyruvate and **14C**-labeled acetate, pyruvate, L-lactate, and butyrate is shown in Fig. 1. No significant cross-inhibition of octanoate or decanoate is observed (Fig. 1).

Cross-inhibition of L-phenylalanine, L-arginine, or D-glucose uptake by pyruvate appears to be negligible (Table 3).

**Discussion**

**Methodology.** The methodology used here is well suited to the study of metabolizable solutes. The penetration of BBB presumably occurs before a significant biotransformation of the labeled compound can occur, since the brain uptake takes place during the first 1–2 sec after injection.

Since the blood in the artery visibly clears during the injection, the bolus of injected solution probably remains with approximately its original chemical composition when it passes through the brain microcirculation. This allows the creation in the injected solution of known concentrations of unlabeled solutes and the measurement of any effects of these unlabeled species on the brain uptake of the radiolabeled species. In this manner, relative affinities of the unlabeled and labeled species for carrier transport sites can be measured. Self-inhibition and cross-inhibition can thus be

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**Table 2. Self-inhibitory effects on brain uptake of 14C organic acids**

<table>
<thead>
<tr>
<th>Organic Acid</th>
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<td>43 \pm 2.1</td>
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<tr>
<td>L-Lactic</td>
<td>0.011</td>
<td>16.2 \pm 1.9*</td>
</tr>
<tr>
<td>Butyric</td>
<td>0.013</td>
<td>46 \pm 4.2</td>
</tr>
</tbody>
</table>

* For each value *n* = 3 except that for L-lactic (0.011 mM), *n* = 12.

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**Fig. 1.** Indicating cross-inhibitory effect on brain uptake of **14C** straight-chain fatty acids with addition of unlabeled pyruvate to solution injected into carotid artery. Short-chain acids show a more prominent degree of saturation than long-chain acids. Greater lipid solubility of latter may mask any carrier-mediated transport they may undergo.
The demonstration that these di- or tricarboxylic acids have electrical charge due to their two or three carboxylic groups, cinate, and fumarate probably relate to their greater total mass (Table 2) and the cross-inhibition by pyruvate of acetate, propionate, and butyrate (Fig. 1) suggest that the uptake of L-lactate were due only to its lipid solubility, whereas the difference between n-lactate, yet it still exhibits an appreciable uptake. If the uptake were lipid mediated, it should be independent of injected concentration. Further support is given to the presence of a specific carrier transport mechanism by the stereospecificity of the uptake of l-lactate. If the uptake of L-lactate were due only to its lipid solubility, it should not be stereospecific, since the lipid solubilities of the l and n enantiomorphs presumably are identical. The lactate stereospecificity here parallels the stereospecificity of brain uptake found after intravenous injection of labeled L- and D-lactate (5).

If the uptakes of only the three-carbon monocarboxylic acids are considered, pyruvate uptake exceeds that of propionate, despite the additional weak hydrogen bonding resulting from the substitution of the oxygen on the center carbon. The hydroxyl group on the center carbon of lactate would be expected to make lactate much more hydrophilic than propionate, yet it still exhibits an appreciable uptake. The very low uptake of D-lactate may represent uptake due to its slight lipid solubility, whereas the difference between L- and D-uptake presumably represents the much greater affinity of the L-enantiomorph for the monocarboxylic carrier system.

The immeasurably low brain uptakes of citrate, succinate, and fumarate probably relate to their greater total electrical charge due to their two or three carboxylic groups, all of which are essentially completely ionized at body pH. The demonstration that these di- or tricarboxylic acids have no measurable uptake supports the specificity of the short-chain organic acid carrier system demonstrated here for monocarboxylic acids.

Uptakes at loc injected concentrations. The increasing brain uptake of the straight-chain, saturated monocarboxylics with increasing chain length would be expected solely on the basis of increasing lipid solubility and is very similar to that found in red cells (3).

In addition to the influence of lipid solubility on uptake, the data presented here suggest that the rate of BBB penetration of the shorter, more hydrophilic acids is greater than that expected on the basis of lipid solubility. That this represents carrier-mediated transport is supported by the partial saturability of the brain uptakes of these shorter chain smaller acids. If the uptake were lipid mediated, it should be independent of injected concentration. Further support is given to the presence of a specific carrier transport mechanism by the stereospecificity of the uptake of l-lactate. If the uptake of L-lactate were due only to its lipid solubility, it should not be stereospecific, since the lipid solubilities of the l and n enantiomorphs presumably are identical. The lactate stereospecificity here parallels the stereospecificity of brain uptake found after intravenous injection of labeled L- and D-lactate (5).

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Uptakes at elevated injected concentrations. The self-inhibition of acetate, pyruvate, butyrate, L-lactate, and propionate (Table 2) and the cross-inhibition by pyruvate of acetate, propionate, L-lactate, and butyrate (Fig. 1) suggest that there is a saturable BBB carrier transport system for which all of these acids have a measurable affinity.

The monocarboxylic carrier system is, from the data contained in Table 2, half saturated at 0.5 mM pyruvate and about 3 mM l-lactate. These concentrations are each 2-4 times their normal blood concentrations in the rat. This indicates the carrier system is approaching a half-saturating state in the presence of concentrations in normal blood of these two anions alone. Other solutes, particularly other short-chain acids, presumably further occupy the BBB carrier sites, suggesting that the transfer rate function of the system could fluctuate quite widely in response to changes in blood acidic anions, particularly pyruvate and lactate. In the presence of a sudden increase in blood lactate in severe exertion, the carrier would become more completely saturated and slow the rate at which the elevated blood lactate could equilibrate with brain.

The failure to demonstrate a cross-competitive effect of pyruvate on octanoate and decanoate uptakes (Fig. 1) probably relates to their high lipid solubility, which would mask any uptake due to specific carrier affinities.

The absence of a cross-inhibiting effect of pyruvate on the uptakes of L-phenylalanine, L-arginine, and D-glucose (Table 3) suggests that these three metabolic substrates penetrate the BBB on transport systems that are independent of the monocarboxylic acid transport carrier demonstrated in the present organic acid studies. Large neutral amino acids (phenylalanine) and basic amino acids (arginine) probably penetrate the BBB on two independent carrier systems (7, 10). The BBB transport of D-glucose is probably independent of amino acid transport (7). These three carrier systems (two for amino acids and one for hexoses) have been the only demonstrated transport systems facilitating the BBB penetration of metabolic substrates. The organic acid studies presented here demonstrate the presence of a fourth, saturable, stereospecific transport system which appears to be, by the method employed, independent of the other three.

Saturable transport of short-chain fatty acids by the intestine has been demonstrated (1, 12). The penetration of red cells by organic acids has been extensively studied (2-4, 11), but specific attempts to demonstrate carrier-mediated transport were unsuccessful (11), although the short-chain monocarboxylic acids used in the present study were not examined.

The teleology of this fourth BBB transport system, which appears to result only in an increased BBB permeability to short-chain monocarboxylic acids, remains obscure. It may function to allow an increased flux of short-chain acids into brain where they may serve as metabolic substrates in normal or pathologic states. Although the present studies only demonstrate facilitated passage of organic acids from blood to brain, the carrier could also function to allow the efflux from brain of lactate in brain in normal and hypoxic states (8).

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REFERENCES