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Kinetic Analysis of the Human Blood–Brain Barrier Transport of Lactate and Its Influence by Hypercapnia

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Summary: Blood–brain barrier permeability to L-lactate was studied in 18 patients with the double indicator technique. Venous outflow curves were obtained during normo- and hypercapnia and were analyzed by means of a model that takes tracer backflux and capillary heterogeneity of transit times into account. The average unidirectional extraction of L-lactate was 15%; the transport from the blood to the brain (PS_1) was 0.081 ml g^{-1} min^{-1} and the transport from the brain to the blood (PS_2) was on the same order of magnitude. In hypercapnia, arterial pH decreased from 7.39 to 7.26 and PS_1 to L-lactate increased significantly by 110%. PS_2 also increased although a statistically significant difference compared to the resting state was not reached. It is concluded that L-lactate is easily taken up by the human brain, and that the mechanism by which it crosses the blood–brain barrier is equilibrative. Furthermore, the brain permeability to lactate is enhanced by hypercapnia and the mechanism is believed to act through the decrease in pH. **Key Words:** Blood–brain barrier—Humans—Lactate—pH dependence—Double indicator technique.

Lactate is a key intermediate in brain ischemia, where it is produced at increased rates, and this may have deleterious effects upon the course of brain infarction. The transport of lactate across the blood–brain barrier (BBB) therefore becomes of major interest. Furthermore, in brain ischemia, tissue pH and maybe also local plasma pH change, and this may effect the transfer rates. The transport kinetics across the BBB in humans has so far not been described. In rats, L-lactate has been shown to cross the BBB by means of facilitated diffusion mediated by a stereospecific monocarboxylic acid carrier (Nemoto and Severinghaus, 1971; Oldendorf, 1972). This carrier permits the escape of lactate from the brain, the brain normally exhibiting a small net efflux of lactate (Drewes et al., 1973).

The present study investigated the bidirectional transport across the human BBB of lactate and the influence of hypercapnia upon this transport.

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Abbreviations used: BBB, blood–brain barrier; BUI, brain uptake index; DTPA, diethylenetnaminepentaacetic acid; ECS, extracellular space; PS, permeability–surface area product.

MATERIALS AND METHODS

Patients

Eighteen patients (mean age of 46 ± 12 years, range of 24 to 71 years) hospitalized for various cerebral disorders requiring carotid angiograms were studied in connection with the angiography after informed consent had been obtained. The study was conducted in agreement with the local ethical committee. Patients with major cerebral lesions (e.g., larger tumors) that might interfere with BBB function were excluded.

After percutaneous punctures in the neck under local anesthesia, using the Seldinger technique a small polyethylene catheter (external diameter of 1.7 mm) was introduced into one of the internal jugular veins. A second catheter (external diameter of 1.2 mm) was introduced into the internal carotid artery on the side appropriate for the angiogram. The tip of the venous catheter was placed in the superior bulb of the internal jugular vein; the tip of the arterial catheter was placed in the internal carotid artery just below the siphon. Correct positioning of the arterial catheter was verified by noting the absence of diffuse facial discoloration after a rapid injection of saline or Evans blue. At the end of the study, which usually lasted 20–30 min, the carotid catheter was used for diagnostic purposes.

Blood–brain barrier permeability measurements

For each determination of BBB permeability, a 1–2 ml bolus containing L-lactate was injected rapidly (1–2 s) through the indwelling intracarotid catheter together with several intravascular reference compounds. Starting a

few seconds before the injection, a continuous series of 1.0 ml blood samples was collected from the venous catheter by means of a sampling machine (Ole Dich Instrumentmakers, Hvidovre, Denmark) and deposited into dry heparinized tubes at a speed of 1 sample/s.

Isotopes

The reference substances contained in each injected bolus were as follows: 10 μCi of $^{24}Na^+$, 40 μCi of [^{113m}In]diethylenetriaminepentaacetic acid (DTPA), and 2 μCi of $^{36}Cl^-$. The test substance used was 10 μCi of ^{14}C-labeled L-lactate. $^{24}Na^+$ was obtained from Risø (Roskilde, Denmark), DTPA from CIS (International CIS, Quentin, France), and the other isotopes from Amersham Corp. (Arlington Heights, IL, U.S.A.) or Du Pont (NEN Research Products, Dreieich, Federal Republic of Germany). The total radiation dose per investigation was 1.36 mSv.

A standard solution was prepared from each bolus by adding an aliquot of injectate to venous blood sampled in a dry heparinized tube before the injection. When repeated studies of the BBB were performed, a time interval of at least 10 min was allowed between measurements in order to decrease isotope background values in the samples.

All isotopes were counted in the plasma of the blood samples, the gamma and beta emitters being counted in the same sample. $^{24}Na^+$ and ^{113m}In were counted in a crystal scintillation counter (Packard autogamma 5385, Packard Instrument Co., Downers Grove, IL, U.S.A.) and appropriate corrections for channel spillover and decay were applied. After decay of the gamma emitters, $^{36}Cl^-$ or ^{14}C was assayed using liquid scintillation counting (scintillation fluid: Instagel; spectrometer; Packard Tricarb 3375) and corrections for quenching and channel spillover were applied using the method of external standardization.

The cerebral blood flow (CBF) was measured during each study using the ^{133}Xe intra-arterial injection method; 3–4 mCi of ^{133}Xe dissolved in 2–3 ml of saline was injected rapidly (1–2 s) into the internal carotid artery through the indwelling catheter. The clearance of the isotope from the hemisphere was followed by an externally positioned array of 16 small scintillation detectors; the average hemispheric blood flow was calculated from the initial slope of the semilogarithmically recorded clearance curves (Olesen et al., 1971). Using a conventional electrode, the arterial carbon dioxide tension, oxygen tension, and pH were determined concomitantly with each CBF measurement. In 9 of the 18 patients, hypercapnia was induced by letting them breathe CO_2.

Calculation of ideal reference curves

All venous outflow curves were normalized by dividing the count rate in each sample by the count rate in the standard solution to obtain an equal total area under the venous outflow curves of reference substances. Ideal reference curves were calculated in all cases in order to avoid the small influence that intravascular separation of test and reference substances has on the relative shape of their venous outflow curves. The intravascular separation phenomena and how to handle these problems have been described in detail elsewhere (Hertz and Paulson, 1980). In all cases, the reference curves were close to the corrected ideal reference curves.

As references for lactate, Na^+ and [In]DTPA were used. Lactate has a molecular size approximately between that of Na^+ and [In]DTPA, so the ideal reference for these substances was calculated as $\frac{1}{2} \cdot (Na^+ +$ [In]DTPA) since lactate, Na^+, and [In]DTPA do not cross the erythrocyte membrane in significant amounts within the experimental period.

Blood–brain barrier models and calculations

For the analysis of the kinetics of the movement of lactate, a mathematical model was used according to which the shape of a theoretical test curve is calculated from that of the reference curve (Fig. 1) (Knudsen et al., 1990*a*). It is assumed that lactate, as D-glucose, within the experimental period instantaneously distributes in the brain extracellular space (ECS) and this volume has previously been found to constitute about 15% (Knudsen et al., 1990*a*). Tracer backflux and heterogeneity of the capillary transit times are taken into account by using a mathematical model. This model assumes, due to the small size of the endothelial cell, that the substance within the endothelial cell is almost immediately in a steady state with respect to the substance within the capillary and the brain ECS. Hence, the endothelial cell behaves as a single membrane. Thus, the parameters characterizing the transfer of substances across the BBB are the permeability of the endothelial cell from blood to the brain ECS (PS_1); the permeability of the endothelial cell from brain ECS to blood (PS_2); and the permeability of the brain cell membranes from the brain ECS into the brain cells (PS_3). In this model, it is assumed that the concentration within the brain cells remains sufficiently small so that there is negligible backflux from the cells to the brain ECS. The equations for the well-mixed model presented in the previous paper (Knudsen et al., 1990*a*) are applicable for this model. Thus, the operational equations for the single membrane model are

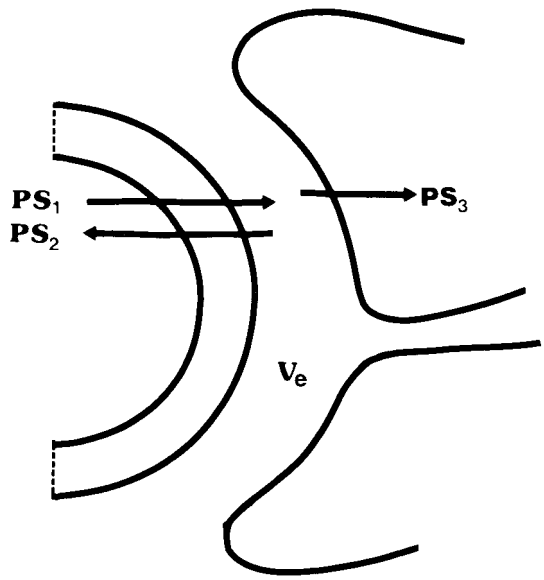

FIG. 1. Model for the blood–brain barrier. PS_1 is the permeability surface area (PS) from the blood to the brain interstitial fluid, PS_2 from the interstitial fluid to the blood, and PS_3 from the interstitial fluid to the intracellular space. V_e is the relative volume of the interstitial fluid.

$$C_{test}(t) = \int_0^{\theta_{max}} C_{ref}(\theta)\, C_c(L, t - \tau_l(\theta), \tau_c(\theta))\, d\theta \quad (1)$$

where $C_{ref}(t)$ and $C_{test}(t)$ are the amounts of the reference and test substances, respectively, as measured at the jugular vein at time t. θ is the total transit time and θ_{max} is the value of θ for which $t - \tau_l(\theta) = 0$, where $\tau_l(\theta)$ is the transit time through the large vessels and $\tau_c(\theta)$ is the transit time through the capillary bed for a given θ. $C_c(y,t,\tau_c)$ is the capillary concentration at position y at time t for a capillary transit time of τ_c and L is the length of the capillary. The Laplace transform of $C_c(L,t,\tau_c)$, i.e., $\bar{C}_c(L)$, is given by

$$\bar{C}_c(L) = e^{-(s+PS_1/V_c)\tau_c} + \frac{\frac{PS_1PS_2\tau_c}{V_cV_e}(1 - e^{-(s+PS_1/V_c)\tau_c}}{\tau_c(s + k_{23})\left(s + \frac{PS_1}{V_c}\right)^2 - \frac{PS_1PS_2}{V_cV_e}\left[\left(s + \frac{PS_1}{V_c}\right)\tau_c - (1 - e^{-(s+PS_1/V_c)\tau_c})\right]} \quad (2)$$

where s is the Laplace transform variable; V_c is the capillary volume; V_e is the volume of the brain ECS; and $k_{23} = (PS_2 + PS_3)/V_e$. The inverse of Eq. (2) is given by

$$C_c(L) = \begin{cases} 0 & (t \neq \tau_c) \\ \delta(0)\, e^{-PS_1\tau_c/V_c} & (t = \tau_c) \end{cases} + \text{the Laplace inverse of the second term of Eq. (2)} \quad (3)$$

where $\delta(O)$ is the Dirac delta bolus input and the Laplace inverse is numerically evaluated (Knudsen et al., 1990*a*).

The values of four variables and their standard errors of the estimates giving the best fit to the measured test curve were obtained by finding the least square of the differences between the theoretical and the measured test outflow curve by means of the simplex method.

Statistics

When repeated studies of BBB permeability for lactate were performed in the same patient, average values were used. For comparison of differences between the calculated variables in normo- and hypercapnia, the paired Wilcoxon's test was applied.

RESULTS

Figure 2 illustrates a representative example of a double indicator curve for L-lactate vs. that of the calculated reference substance.

Table 1 summarizes the variables obtained for L-

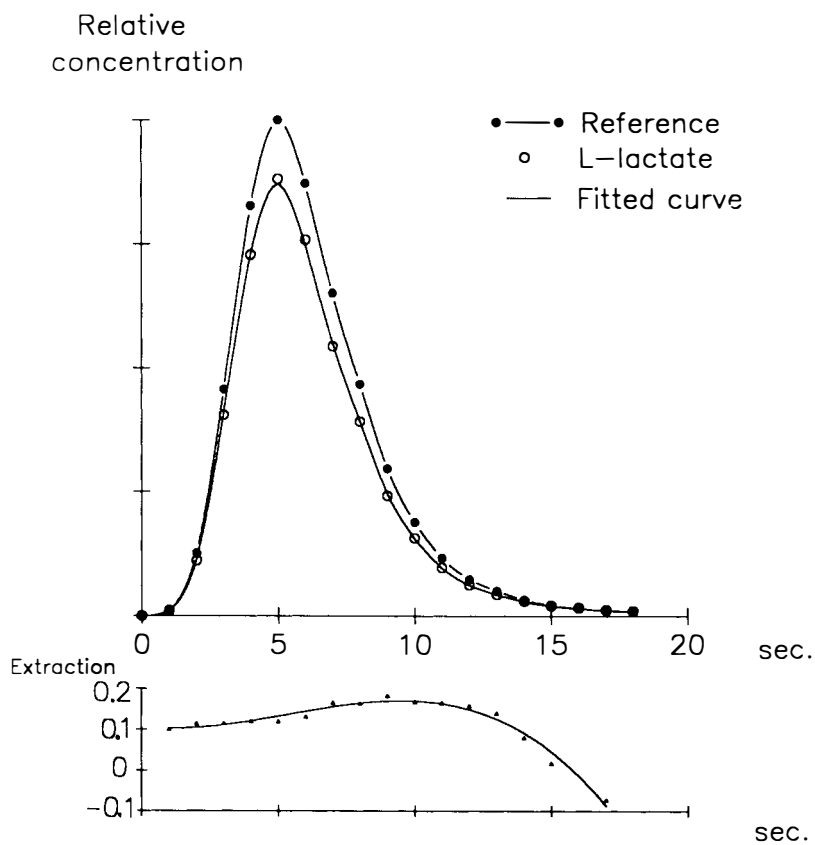

FIG. 2. Venous outflow curve and corresponding extraction curve for L-lactate.

lactate in the resting state. The permeability–surface area product (PS) for transport from the blood to the brain (PS_1) amounts to 0.08 ml g^{-1} min^{-1}. Within an age range of 24 to 71 years in our patients, there was no correlation between PS_1 and age. Assuming that L-lactate, as D-glucose, distributes in the ECS, then the permeabilities out of the brain back to the blood (PS_2) and the intracellular uptake (PS_3) are calculated to be in the same range as PS_1. The unidirectional average extraction E is 15%.

Arterial blood gas variables and CBF in normocapnia versus hypercapnia are shown in Table 2. In hypercapnia, arterial $Paco_2$ increased from 37 to 57 mm Hg, and arterial pH dropped from 7.39 to 7.26. $Paco_2$ in blood from the jugular vein was ~5% higher than in the arterial blood, signifying that a CO_2 saturation of the brain tissue was approaching. CBF was almost doubled; it increased from 43 to 83 ml (100 g^{-1} min^{-1}). The transport variables of L-lactate across the BBB in hypercapnia compared to normocapnia are shown in Table 3. PS_1 increased significantly with decreasing pH, from 0.084 to 0.176 ml g^{-1} min^{-1}. PS_2 increased from 0.095 to 0.131 and PS_3 from 0.130 to 0.383 ml g^{-1} min^{-1}, but these differences were not statistically significant and the scatter of PS_3 determinations was large. The extraction E remained at the baseline level.

DISCUSSION

Lactate versus D-glucose

In the present study, a considerable permeability of lactate across the human BBB is demonstrated. PS_1 for L-lactate is about one-half that for D-glucose, i.e., 0.08 versus 0.15 ml g^{-1} min^{-1} (Knudsen et al., 1990*a*). This PS_1 value agrees well with the one of 0.06 ml g^{-1} min^{-1} that was found by Pardridge and co-workers in rats (Pardridge et al., 1975). Oldendorf and later Alm and Törnquist found by means of the brain uptake index (BUI) technique with water as the diffusible tracer a BUI of 16%, i.e., in the same range (Oldendorf et al., 1979; Alm and Törnquist, 1985). The constant level of PS_1 for L-lactate in adults agrees with the findings of Cremer, who found that lactate uptake decreases with age but remains relatively constant in adult rats (Cremer et al., 1976, 1979).

Assuming that L-lactate has the same distribution space as D-glucose, PS_2 is similar to PS_1. Similar values for PS_1 and PS_2 were expected since there has been no evidence for active transport across the BBB as may be the case, for example, for amino acids (Knudsen et al., 1990*b*). If C_p is the plasma capillary and C_b the ECS concentration of lactate, then the brain influx equals $PS_1 \cdot C_p$ and the efflux is $PS_2 \cdot C_b$, i.e., the ratio of efflux to influx equals the relative lactate concentrations of ECS and plasma as long as the transport system is not saturated on either side of the BBB. The brain efflux of lactate only doubles when ECS lactate concentration in dogs increases to nine times the normal level and this may be a sign of saturation of the monocarboxylic carrier (Drewes et al., 1973). The K_m for carrier transport across the BBB has in animals been estimated to be about 2.6 m*M* (Pardridge et al., 1975), suggesting that saturation may occur at high lactate concentrations.

An example of the order of magnitude for the fluxes across the BBB will be given. The plasma concentration of L-lactate in humans is about 1.0 m*M* and the ECS lactate concentration is about 1.5 m*M* (Lentner, 1984). Using these values and our PS_1 value from Table 1 yields an influx of about 80 nmol g^{-1} min^{-1}, an efflux of 120 nmol g^{-1} min^{-1}, and thus a net brain efflux of 40 nmol g^{-1} min^{-1}. This net efflux is on the same order of magnitude as the one reported in dogs (Drewes and Gilboe, 1973). The finding of almost equal influx and efflux at nor-

TABLE 1. *Blood–brain barrier transport parameters for L-lactate in 18 humans in the resting state*

n		18
PS_1	(ml g^{-1} min^{-1})	0.082 ± 0.033
PS_2	(ml g^{-1} min^{-1})	0.105 ± 0.182
PS_3	(ml g^{-1} min^{-1})	0.091 ± 0.172
E		0.154 ± 0.050

Values are means ± SD for the whole group.
n is number of patients, E is the unidirectional extraction.

TABLE 2. *Arterial blood gas parameters and cerebral blood flow in nine patients subjected to two conditions*

	pH	$PaCO_2$ (mm Hg)	PaO_2 (mm Hg)	CBF g^{-1} (ml 100 min^{-1})
Normocapnia	7.39 ± 0.02	37.4 ± 3.4	80 ± 15	43 ± 11
Hypercapnia	7.26 ± 0.04	57.4 ± 4.3	105 ± 9	83 ± 35

Values are means ± SD for the whole group.

TABLE 3. *Blood–brain barrier transfer variables for L-lactate in nine patients subjected to two conditions*

		Normocapnia	Hypercapnia
n		9	9
PS_1	(ml g^{-1} min^{-1})	0.084 ± 0.037*	0.176 ± 0.135
PS_2	(ml g^{-1} min^{-1})	0.095 ± 0.024	0.131 ± 0.159
PS_2/PS_1		1.13	0.74
PS_3	(ml g^{-1} min^{-1})	0.130 ± 0.248	0.383 ± 0.802
E		0.164 ± 0.056	0.172 ± 0.088

Values are means ± SD for each group.
* $p < 0.01$.

mal concentrations of lactate, however, is not consistent with another study in rats, where the rate of lactate efflux from brain by means of the BUI technique was estimated to be about three times that of the influx (Pardridge et al., 1975). This discrepancy is obscure but differences in the applied techniques or species differences may contribute.

With the present data, the intracellular uptake PS_3 is larger for L-lactate than for D-glucose (Knudsen et al., 1990*a*). It has recently been found that L-lactate also crosses the neuronal membrane by means of facilitated diffusion (Nedergaard, personal communication) and a rapid bidirectional exchange across this cell membrane may facilitate the escape of lactate from neurons.

pH dependency

The present data show that PS_1 for lactate increases in hypercapnia. This increase could be caused either by an increase in the capillary surface area or by an increase in lactate permeability. An increased capillary surface area could have been a consequence of the CBF increasing. This hypothesis is less likely since, in hypercapnic rats, the relationship between regional CBF and the regional density of perfused capillaries shows that while regional CBF values are significantly increased, the regional densities of perfused capillaries remain unchanged (Göbel et al., 1989). Furthermore, in a previous study, we found evidence that the capillary heterogeneity decreased with increasing CBF but there was no evidence for an increase in the surface area (Knudsen et al., 1990*a*). It therefore seems more likely that the decreased pH is responsible for increasing the BBB permeability to lactate in the present study. Additional support comes from animal experiments where the brain uptake of L-lactate, as measured by the BUI technique, decreases with increasing pH (Oldendorf et al., 1979; Alm and Törnquist, 1985).

The unionized fraction of the total amount of an acid C_u/C_t, is determined by

$$C_u/C_t = 1 / (1 + 10^{(pH - pK_a)}) \quad (3)$$

The unionized fraction of lactate is assumed to cross the lipophilic membrane of the BBB by means of simple diffusion, whereas the ionized fraction of L-lactate probably is transported across the BBB by means of the specific monocarboxylic carrier. Since the pK_a for lactate is 3.83, and pH in the present study in normocapnia was about 7.39 and in hypercapnia was 7.26, it follows that only 0.0275% of lactate is in the unionized form at normal pH. In hypercapnia, the concentration of unionized lactate increases by 35%, but since the major part of L-lactate transport is managed by a specific carrier (Cremer et al., 1979), the increase in the unionized fraction is without major significance and can not account for the PS_1 increase of 110%. We therefore find it more likely that decreasing pH has an activating effect upon the lactate carrier located at the BBB. That carrier systems at the BBB are influenced by plasma pH is known, for example, from the transport of D-glucose, which increases with increasing pH (Hertz and Paulson, 1982). From a teleological point of view, it is beneficial that the escape of lactate is facilitated in the case of decreased interstitial pH.

In conclusion, the BBB permeability to L-lactate is in humans about one-half that of D-glucose. The transport across the BBB takes place by means of facilitated diffusion and this equilibrative transport has implications for conditions with increased ECS concentrations of lactate. Finally, the brain permeability surface area to lactate is highly influenced by hypercapnia, which enhances the brain uptake of lactate. This increase in PS_1 is explained as being caused primarily by a direct pH effect upon the carrier and not by an increase in the capillary surface area.

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