D-Lactic acid-induced neurotoxicity in a calf model

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D-Lactic acid-induced neurotoxicity in a calf model. Am J Physiol Endocrinol Metab 293: E558–E565, 2007. First published May 15, 2007; doi:10.1152/ajpendo.00063.2007.—Lactic acidosis (DAC) occurs as a complication of short-bowel syndrome in humans and in a variety of other gastrointestinal disorders in monogastrics and ruminants. DAC is associated with signs of impaired central nervous system function including ataxia and coma. The objective of this experiment was to determine whether either acidification of nervous tissue or D-lactic acid is responsible for decreased neurological function. Eight Holstein calves (32 ± 11 days, 70 ± 10 kg) were surgically catheterized with indwelling intravenous jugular and atlanto-occipital space cerebrospinal fluid (CSF) catheters and infused for 6 h in random order with isomolar D-lactic acid (DL-LA), L-lactic acid (L-LA), hydrochloric acid (HCl), or saline. DL-LA induced ataxia after 4 h of infusion and produced the greatest obtunding of CNS function (at 7 h, score 8.0 ± 0.4), whereas the other infusions caused neither ataxia nor scores over 1.5 (P < 0.01 from DL-LA). DL-LA induced significantly less acidemia than HCl (at 6 h pH 7.13 ± 0.06 and 7.00 ± 0.04, base excess −16 ± 1 and −23 ± 3 mmol/l, bicarbonate 11 ± 1 and 8 ± 1 mmol/l respectively, all P < 0.01) but greater than L-LA and saline (P < 0.01). CSF changes followed a similar but less pronounced pattern. Although HCl infusion produced a severe acidemia and CSF acidosis, only minor effects on neurological function were evident suggesting that D-lactate has a direct neurotoxic effect that is independent of acidosis. Conversely, L-LA produced only minor neurological changes. L-lactic acid; hydrochloric acid; acidosis; cerebrospinal fluid; clinical signs

ACIDEMIA IS A COMMON PROBLEM in humans and other animals. In diarrheic calves, the severity of acidemia correlates with neurological depression (41). In humans with short-bowel syndrome, D-lactic acidosis is suggested as the cause of certain neurological symptoms (4–6, 27, 28, 43, 49, 64). Similarly, in a variety of ruminant diseases, including neonatal diarrhea, ruminal carbohydrate overload, and acidosis without dehydration syndromes, D-lactic acidosis occurs in association with impaired central nervous system function, including signs of ataxia and coma (16, 19, 21, 31, 47, 53, 60). In cats, D-lactic acidosis occurs in experimental propylene glycol intoxication (10) and in exocrine pancreatic insufficiency (48). In both cases, D-lactic acidosis is associated with CNS depression, ataxia, and in one case coma. In a recent human case, accidental propylene glycol ingestion was associated with a very high serum D-lactate concentration (110 mmol/l), subsequent loss of CNS function, and development of coma (31).

At physiological pH, D-lactic acid is almost completely dissociated (15, 18). Whether or not acidosis per se or the D-lactate anion is responsible for impaired CNS function is not well established. Injection of a bolus of 223 mmol sodium D-lactate in 100 ml of water produced ataxia, depression, and a reduced palpebral reflex in calves (37). However, the experiment was criticized, since the solution was hyperosmolar, with an osmotic pressure ~15 times that of plasma; therefore, some of the neurological signs might have been related to fluid shifts (57). Similarly, there is disagreement about whether D-lactate is directly responsible for neurological signs in humans (64).

The objectives of this experiment were to compare the influence of different types of acidosis on neurological function and to determine whether neurological disturbances are caused by either increased acidity or D- or L-lactate concentrations.

MATERIALS AND METHODS

Experimental Animals and Care

Calves for this study were obtained from the Dairy Barn of the Department of Animal and Poultry Sciences, University of Saskatchewan, Canada (Table 1). The Holstein calves were housed indoors in the Animal Care Facility at the Western College of Veterinary Medicine, University of Saskatchewan. The indoor environment was held at 22 ± 1°C, 65 ± 5% relative humidity and was lit from 600 to 2000. Each calf was housed in a 2.0 × 1.5-m2 pen. Flooring was a 3-cm-thick rubber mat covered with ~4 cm of wood shavings.

Clean water was freely available to these calves through automatic water bowls fixed to the walls. The stalls were cleaned twice a day. The calves were offered alfalfa hay and fed milk replacer (20 - 20 - 20 Wet Nurse, Green Tag; Prairie Micro-Tech, Regina, Saskatchewan, Canada) twice a day. Milk replacer solution was made on a 20% vol/vol basis and fed at 2.5–3.0 liters a meal. The calves were weighed (Norac Instaweigh Instrumentation Animal Scale; Norac Weighing and Control Systems, Norac International, Saskatoon, Saskatchewan, Canada) on the arrival to the Animal Care Facility, and prior to the catheterization (Table 1). The latter weight was used for dosage calculations.

Catheterization

Calves were catheterized with indwelling jugular intravenous, ear arterial, and atlanto-occipital space cerebrospinal fluid (CSF) catheters (12, 14, 30). All surgeries were performed in the Department of Large Animal Clinical Science, Western College of Veterinary Medicine, University of Saskatchewan. The calves were premedicated with intravenous hydromorphone at 0.1 mg/kg, sometimes in combination with metamizol at 7 μg/kg or medetomidine at 4 μg/kg, induced with a combination of ketamine at 2–6 mg/kg and diazepam at 0.1–0.5 mg/kg, intubated, and maintained under gaseous isoflurane anesthesia. Strict aseptic technique was followed throughout the catheter placement procedure.

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Table 1. Calf information and order of infusion of DL-LA, HCl, t-LA, and saline

<table>
<thead>
<tr>
<th>Calf ID</th>
<th>Body Weight, kg</th>
<th>Age, days</th>
<th>Order of Infusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>6458588504</td>
<td>62</td>
<td>35</td>
<td>2*</td>
</tr>
<tr>
<td>287348803502</td>
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<td>1</td>
</tr>
<tr>
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<td>17</td>
<td>1</td>
</tr>
<tr>
<td>8645724888</td>
<td>68</td>
<td>15</td>
<td>1</td>
</tr>
</tbody>
</table>

Total No. of infusions: 6 5 6 6

Mean ± SE: 69.6 ± 3.6 32.0 ± 3.9

*Numbers indicate infusion order: 1, infused first; 2, second infusion; 3, third infusion; 4, fourth infusion. LA, lactic acid; NA, infusion not performed.

**Experimental Design**

Eight calves randomly received intravenous infusions of DL-LA, t-LA, HCl, or saline (Table 1) at an infusion rate of 20 ml/kg body wt \(^{-1}\) h\(^{-1}\) using an automated infusion pump (Imed 980 volumetric infusion pump; Imed, San Diego, CA) for 4 h. Infusion was continued for a further 2 h until venous pH reached 7.05. If pH rose above 7.1 infusion was restarted. After 12 h from the beginning of the infusion, severely acidic calves were treated with 155 mmol/l NaHCO\(_3\) solution (Sigma-Aldrich, St. Louis, MO) to correct blood pH and alleviate neurological disturbances. There was a minimum period of 2 days between each infusion.

**Sampling and Measurements**

Samples of CSF fluid (~0.7 ml) and venous (4 ml) and arterial (1 ml) blood were collected, and neurological assessment was performed at -1 (preinfusion), 0 (start of infusion), 1, 2, 3, 4, 5, 6 (completion of infusion), 7 (postinfusion), 8, 10, and 24 h. Neurological assessment was performed by a veterinarian who was blinded to the type of infusion being administered. Neurological function was scored on the basis of the strength of the suck, menace, palpebral, and tactile reflexes and ability to stand (34, 38, 41). Each item was given a score of from 0 (normal) to 2 (severely abnormal) according to a previously published scale (34). The values were summed to produce a total neurological score (TNS). Calves with normal CNS function had scores of 0, whereas severely obtunded calves could have a maximum score of 10.

**Laboratory Analyses**

Heparinized arterial and venous blood (1 ml each) and 0.3 ml of CSF fluid were placed on ice and analyzed within 40 min of collection for pH, bicarbonate (HCO\(_3^\)-), base excess, partial pressure of carbon dioxide and oxygen (PC\(_{O_2}\) and PC\(_{O_2}\)) using a blood gas analyzer (ABL 5; Radiometer Medical, Copenhagen, Denmark) (55). Hemoglobin and its oxygenation status were measured in a hemoximeter (OSM3; Radiometer Medical) (68). The remaining venous blood (3 ml) was allowed to coagulate for 20 min, and serum was separated in a refrigerated centrifuge (IEC Centra-7R Refrigerated Centrifuge; International Equipment, Needham Heights, MA) at 1,000 RCF for 30 min. The serum and remaining CSF fluid were stored at -20°C. t-LA and DL-LA concentrations were measured using an HPLC system (Waters 600 HPLC pump, 486 UV detector, 786 Ultra WISP autoinjector, and Millenium v. 4 software; Waters, Mississauga, ON, Canada) as we previously described (46). For the separation of t-LA and dl-LA a 3-μm ODS-packed (50 mm × 4.6 mm) analytical column (Waters Guard-Pak precolumn; Waters), coated with N,N-diocetyl-alanine (ChiralPak MA+; Chiral Technologies, Exton, PA) was employed (46).
Infusate pH was measured using a pH meter (Beckman Ø 32 pH meter; Beckman Instruments, Fullerton, CA).

Statistical Analysis

Graphs and figures were created using a spreadsheet (Microsoft Excel; Microsoft, Redmond, WA). Descriptive statistics and Pearson correlation coefficients were calculated using a statistical analytical software package (SPSS statistical software v. 13, 2005; SPSS, Chicago, IL). Analysis of variance (ANOVA) with repeated measures was carried out using SAS statistical software (SAS/STAT statistical software package for Windows, v. 8.02; SAS Institute, Cary, NC). The significance of treatment effects on neurological scores, blood gas values, and serum and CSF lactates was determined by repeated-measures ANOVA using treatment (infusion) as the independent variable (59). Mean values at individual time points were compared using the Student-Newman-Keuls (SNK) test. Treatment differences were also tested using orthogonal contrasts to compare DL-LA vs. HCl, L-LA, and saline. A P value of ≤0.05 was considered to be statistically significant. Values are reported as means ± SE.

RESULTS

All infusions were continued for 6 h except for HCl, which was given intermittently between hours 4 and 6 to maintain venous pH above 7.0 and below 7.10.

Neurological Assessment

Saline had no effect on neurological score (Fig. 1). L-LA and HCl infusions produced mild impairments in neurological function and had no significant effect on the menace, tactile, and palpebral reflexes at any time period. DL-LA-infused calves had a significantly reduced menace reflex between 1 and 10 h; the menace response was completely absent at 6, 7, and 8 h of infusion. Ability to stand was significantly inhibited by DL-LA infusion between 2 and 10 h; weakness was detected at 2 h, developed into ataxia at 4 h, and progressed to involuntary recumbency at 8 h. During infusions other than DL-LA, involuntary recumbency was only seen on one occasion, in a calf receiving L-LA. In DL-LA-infused calves, significant reductions in the palpebral and tactile reflexes were first detected at 3 h, increased to maximum at 7 or 8 h, and slightly recovered by 10 h. The suck reflex was unusual in that HCl and DL-LA similarly depressed this reflex; L-LA had a smaller effect (Fig. 2).

Acidosis

HCl infusion produced the most severe metabolic acidosis, which reached a nadir at 4 h of infusion; venous blood pH 6.9 ± 0.1, bicarbonate 7.00 ± 1.45 mmol/l, and base excess (BE) −23.3 ± 1.7 mmol/l. DL-LA produced a significantly less severe acidemia than HCl; at 6 h, pH 7.13 ± 0.06 and 7.00 ± 0.04, bicarbonate 11 ± 1 and 8 ± 1 mmol/l and base excess −16 ± 1 and −23 ± 3 mmol/l for DL-LA and HCl, respectively, all P < 0.01. In contrast, L-LA infusion resulted in little change in acidemia with blood gas values similar to those during saline infusion (Table 2).

Both HCl and DL-LA infusions produced similar decreases in CSF pH (Fig. 3), bicarbonate (Fig. 4) and PCO2 (Table 2). Arterial blood bicarbonate (r = 0.87), BE (r = 0.84), and PCO2 (r = 0.78) were highly correlated (P < 0.01) with the corresponding CSF measurements. However, arterial and CSF pH were only moderately correlated (r = 0.57). The correlations between CSF and venous blood gas measurements were not as strong; correlations for CSF and venous blood measurements of bicarbonate, PCO2 and pH were 0.85, 0.56, and 0.46 respectively.

DL- and L-Lactate Concentrations

Serum and CSF D-lactate were increased only by DL-LA infusion. Venous serum and CSF D-lactate concentrations were highly correlated (r = 0.91, P < 0.01). However, changes in CSF D-lactate lagged behind those in serum (Fig. 5).

Serum L-lactate concentrations were unaffected by HCl infusion, increased to a moderate extent during DL-LA infusion, and were highest during L-LA infusion. CSF L-lactates were also highest with L-LA infusion and moderately increased with DL-LA infusion (Table 2). During L-LA infusion, peak CSF L-lactate concentrations were attained at 6 h (11 ± 1 mmol/l)
Table 2. Effects of d-LA, L-LA, HCl, or saline infusions on arterial, venous and cerebrospinal fluid blood gas values, and cerebrospinal fluid and venous serum lactate concentrations

| Infusion   | d-LA               | L-LA               | HCl        | Saline
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>d-Lactate, mmol/l</td>
<td>23.5±2.2</td>
<td>1.1±0.2</td>
<td>0.9±0.1</td>
<td>0.6±0.1</td>
</tr>
<tr>
<td>L-Lactate, mmol/l</td>
<td>4.5±0.4</td>
<td>8.5±0.5</td>
<td>1.7±0.2</td>
<td>1.8±0.1</td>
</tr>
</tbody>
</table>

**Serum**

| pH         | 7.21±0.02          | 7.33±0.02          | 7.05±0.02  | 7.35±0.01 |
| Base excess, mmol/l | −11.1±1.0          | 0.4±0.9            | −18.6±1.0  | 1.7±1.0   |
| HCO₃⁻, mmol/l      | 15.6±0.6           | 25.3±0.9           | 10.1±0.5   | 26.5±0.9  |
| PO₂, mmHg           | 40.3±1.4           | 48.0±1.9           | 34.2±1.7   | 49.7±1.5  |
| Po₂, mmHg           | 44.25±1.21         | 39.54±1.82         | 41.39±2.28 | 36.36±1.25|
| Hemoglobin, g/l     | 82.92±9.21         | 74.08±6.52         | 85.67±8.93 | 75.56±7.84 |

**Venous blood**

| pH         | 7.26±0.02          | 7.38±0.02          | 7.08±0.02  | 7.41±0.01 |
| Base excess, mmol/l | −11.1±1.0          | −3.0±0.9           | −20.2±0.9  | 2.8±1.6   |
| HCO₃⁻, mmol/l      | 14.3±0.1           | 20.9±1.1           | 7.4±0.7    | 26.6±1.9  |
| PO₂, mmHg           | 32.8±1.7           | 36.8±1.7           | 25.2±1.9   | 44.1±2.9  |
| Po₂, mmHg           | 107.1±5.3          | 105.0±7.2          | 120.2±5.5  | 95.3±6.9  |
| Hemoglobin, g/l     | 71.8±10.0          | 62.3±6.7           | 74.5±9.1   | 61.8±13.3 |

**Arterial blood**

| pH         | 7.24±0.04          | 7.29±0.02          | 7.20±0.06  | 7.29±0.02 |
| Base excess, mmol/l | −10.2±0.9          | −20.2±0.9          | −20.2±0.9  | −20.2±0.9 |
| HCO₃⁻, mmol/l      | 18.3±0.9           | 22.5±0.9           | 163±1.8    | 24.2±1.7  |
| PO₂, mmHg           | 44.9±2.5           | 49.5±2.5           | 43.4±3.9   | 53.3±1.9  |
| Po₂, mmHg           | 80.9±1.6           | 73.2±3.8           | 81.7±3.5   | 83.1±2.9  |

**CSF**

| d-Lactate, mmol/l | 14.8±3.8          | 0.2±0.1            | 0.2±0.1    | 0.04±0.01 |
| L-Lactate, mmol/l  | 6.9±1.2           | 10.7±0.8           | 3.8±1.0    | 3.4±0.5   |
| pH                 | 7.24±0.04         | 7.29±0.02          | 7.20±0.06  | 7.29±0.02 |
| HCO₃⁻, mmol/l      | 18.3±0.9          | 22.5±0.9           | 163±1.8    | 24.2±1.7  |
| PO₂, mmHg           | 44.9±2.5          | 49.5±2.5           | 43.4±3.9   | 53.3±1.9  |
| Po₂, mmHg           | 80.9±1.6          | 73.2±3.8           | 81.7±3.5   | 83.1±2.9  |

Values are means ± SE for data collected between 1 and 7 h of infusion. Statistical significance was determined for values between 1 and 7 h of infusion inclusive by repeated-measures ANOVA and orthogonal contrast comparison for d-LA vs. HCl or L-LA treatments.

and were slightly higher than peak serum L-LA concentration (10 ± 1 mmol/l). During DL-LA infusion, CSF L-LA concentration (8 ± 1 mmol/l) peaked at 6 h and was 60% higher than serum concentration (5 ± 2 mmol/l). During DL-LA infusion, peak serum and CSF L-LA concentrations were considerably lower than those of peak D-lactate concentrations (Table 2).

**Clearance of d-lactate**

Following cessation of infusion, serum d-lactate concentrations declined in an exponential fashion with time (r = 0.98, P < 0.01). Clearance of d-lactate from CSF was slower and

![Fig. 3. Change in cerebrospinal fluid (CSF) pH values during (0 – 6 h) and after infusion of dl-LA, l-LA, HCl, or saline. Values at 24 h returned to baseline. Repeated-measures analysis showed significant effects of treatment (P < 0.01), time (P < 0.0001), and treatment vs. time interaction (P < 0.0006). Values at each time point that do not share a common letter (a, b, or c) are statistically significant at P < 0.01 by Student-Newman-Keuls test.](http://ajpendo.physiology.org/)

![Fig. 4. Change in CSF bicarbonate concentrations during (0 – 6 h) and after infusion of dl-LA, l-LA, HCl, or saline. Values at 24 h returned to baseline. Values at each time point that do not share a common letter (a, b, or c) are statistically significant at P < 0.01 by Student-Newman-Keuls test.](http://ajpendo.physiology.org/)
more gradual than from serum (Fig. 5). CSF clearance was constant over the 12-h postinfusion period, whereas clearance from serum declined with time and correlated \((r = 0.73, P < 0.01)\) with serum \(\text{D-lactate}\) concentration.

**Correlation of Neurological Disturbances with Changes in Blood and CSF Chemistry**

CSF and serum \(\text{D-lactate}\) concentrations strongly correlated \((P < 0.01)\) with all neurological scores except for the strength of the suck reflex (Table 3). The suck reflex was affected similarly by both \(\text{DL-LA}\) and \(\text{HCl}\) infusions (Fig. 2) and was more significant, of the suck reflex (Table 3). The suck reflex was affected \((P < 0.0001)\), time \((P < 0.0001)\), and treatment vs. time interaction \((P < 0.0001)\) for both serum and CSF concentrations.

**DISCUSSION**

\(\text{D-lactic acidosis has been associated with neurological dysfunction in a variety of species, including humans, cattle, cats, and dogs (31, 48, 50, 64). The reported signs in humans include slurred speech, loss of reflexes, abnormal gate, ataxia, paresis, and sometimes coma. In calves (37) and cats (48), impairment of palpebral and menace reflexes have been reported in association with other neurological disturbances. Although clinical studies have consistently shown an association between \(\text{D-lactate}\) concentration and neurological disturbance, a causal link has not been established (28, 31, 48, 50, 64, 67). In many of these studies, other parameters, such as acidemia, were also associated with CNS impairment. In our study, severe acidemia with only mild impairments of neurological function were induced by \(\text{HCl}\) infusion, whereas \(\text{DL-LA}\) infusion produced less acidemia but severe neurological derangements (Fig. 1). The derangements observed with \(\text{DL-LA}\) are similar to those reported clinically for \(\text{D-lactic acidosis and include decreased palpebral and menace reflexes, ataxia, involuntary recumbency, and a comatose appearance. This toxicity is independent of changes in blood or CSF pH, because HCl infusion produced a more severe metabolic acidosis and a similar CSF acidosis but only small changes in neurological function. Toxicity is also unrelated to the \(\text{L-LA}\) component of the \(\text{DL-LA}\) infusion as infusion of pure \(\text{L-LA}\) produced a more marked \(\text{l-lactatemia but had only small effects on neurological function. Thus, D-lactate was the neurotoxic agent in our study. The majority of the infused d-lactic acid would dissociate into D-lactate and hydrogen ions in the blood stream. The conclusion that D-lactate is neurotoxic is supported by the high correlation between CSF \(\text{D-lactate}\) concentrations and neurological disturbances (Table 3). A secondary role for \(\text{L-LA}\) cannot be completely excluded, as this was also infused. L-LA has been previously shown to affect d-lactic acid metabolism (11). However, our inference that \(\text{D-lactate}\) is neurotoxic is consistent with earlier work in which signs of ataxia, somnolence, and impaired palpebral reflexes developed following hypertonic sodium \(\text{D-lactate}\) injection in calves (37). Our experiment also indicates that \(\text{D-lactate}, not hypertonicity as some have proposed (57), is the toxic agent. D-Lactate may be**
transported into neurons with either sodium or hydrogen ions via Na\(^+\)- or H\(^+\)-monocarboxylate transporters (39, 56). Hence, either D-lactate or D-lactic acid may be the toxic agent intracellularly.

In previous experiments, oral administration of DL-LA did not significantly influence humans physiologically or pathologically (15). This lack of effect can be explained by the much smaller amounts of DL-LA administered, a maximum of 12.8 mmol/kg\(^{0.75}\) of racemic DL-LA per day. The total dosage administered in the present experiment was eight times higher. Consequently, plasma D-lactate did not exceed 1 mmol/l in the previous experiment, whereas we obtained peak serum concentrations of \(\sim 36\) mmol/l. Clinical reports of neurological dysfunction suggest that t-lactate concentrations would have to be at least 3 mmol/l before clinical signs are seen (64).

In the present study, the suck reflex (Fig. 2) was correlated more strongly with CSF bicarbonate, BE, and pH than with CSF D-lactate concentration (Table 3). Similarly, clinical studies in calves have noted that ataxia and loss of the palpebral reflex are correlated with D-lactate concentrations whereas the strength of the suck reflex is correlated with acidemia (36). Taken together, this work suggests that acidemia depresses the suck reflex but is not responsible for ataxia or changes in the palpebral reflex.

In the present study, neurological depression was first observed at 2 h of DL-LA infusion when mean serum D-lactate was 16 \(\pm\) 1 mmol/l, which is higher than the serum D-lactate concentrations of \(\sim 3\) mmol/l reported in some clinical cases (64). Our previous studies established that diarrheic calves with neurological depression have serum D-lactate concentrations as high as 26 mmol/l (17, 47). The serum D-lactate concentrations in our current infusion study are well below the serum value (110 mmol/l) reported in a patient intoxicated after overingestion of propylene glycol (31). Furthermore, our data show that it takes time for D-lactate to diffuse into the CSF. At 1 h of infusion, mean serum D-lactate was 11 \(\pm\) 1 mmol/l but CSF D-lactate was 2 \(\pm\) 1 mmol/l. It is possible that a more gradual infusion would result in greater equilibration between CSF and blood D-lactate concentrations with clinical signs at lower venous serum D-lactate concentrations.

In the brain, astrocytes are net producers of L-lactate, because their arms are too thin to accommodate mitochondria (26, 29). The L-lactate is metabolized by neurons (2, 7, 29, 40, 54). It has been estimated that 75% of neuronal oxygen consumption is accounted for by L-lactate metabolism and 25% by glucose metabolism (7). Lactate utilization may be particularly important during acidemia, since low pH can inhibit phosphofructokinase activity, a key glycolytic regulatory enzyme (35). In neurons, uptake of L-lactate is close to saturation at physiological L-lactate concentrations (25). D-Lactate blocks the ability of isolated optic nerves to generate an action potential, probably by competitively blocking L-lactate entry into neurons and limiting neuronal metabolism (61, 62). L-lactate can act as an energy source during ischemia-reperfusion injury and reduces cerebral injury (9, 52). In contrast, D-lactate enhances neuronal injury in ischemia-reperfusion models (9). Although, D-lactate dehydrogenase is found in mammalian mitochondria, particularly in liver and kidney, it is poorly expressed in human brain tissue (20). It is therefore possible that the neurotoxic effects of D-lactic acid infusion observed in the present study are the result of reduced L-lactate availability within neurons and energy deficit. In support of this, the serum-CSF L-lactate difference was slightly greater during DL-LA infusion; this may reflect reduced neuronal removal of astrocyte produced L-lactate (Table 2). Similarly, others have shown that blockade of neuronal monocarboxylate transporters responsible for L-lactate entry results in increased neuronal cell death in the developing brain (1).

Rapid ethanol consumption is another situation wherein acute neurological disturbances are observed in humans (24). We were impressed with the “drunken” appearance of our calves during DL-LA infusion. In acute ethanol consumption, neurological depression may be caused by low concentrations of circulating tryptophan (3) or interactions with the GABA\(_{\text{A}}\) receptor complex and facilitation of GABA action (23, 58). However, ethanol also has early effects on energy metabolism (1, 33, 65), which may be similar to some of the actions of D-lactate.

In addition to lipid-mediated penetration (45), L-lactate may be transported across the blood-brain barrier by monocarboxylic acid transporters (22, 25, 54), which are stereospecific (61, 62). During DL-LA infusion, CSF L-lactate concentrations increased steadily and were higher than serum concentrations by a similar amount in all infusions (Table 2). L-Lactate was probably being formed within nervous tissue by metabolism of glucose, and this likely contributes to the higher concentrations in CSF (2, 22). In our experiment, D-lactate rapidly crossed the blood-brain barrier, although CSF concentrations were about one-half those of serum (Table 2). This could be explained by serum L-lactate competing with serum D-lactate for uptake from blood by CSF tissue (61, 62).

In the present study and in previous work (37), serum D-lactate exhibited an exponential decay. However, CSF D-lactate clearance was constant over the period studied. Although D-lactate infusion was stopped at 6 h, serum D-lactate concentrations were still higher than those in CSF; this likely resulted in some transfer into the CSF confounding the calculations of clearance. At 10 h, CSF D-lactate concentrations were higher than in serum; the slow disappearance of CSF D-lactate would explain the prolonged neurological disturbance and encephalopathy observed in many clinical D-lactic acidosis cases even after treatment has been initiated (50, 51, 64). Monocarboxylic transporters may remove D-lactate from CSF (61, 62). D-Lactate in blood may be taken up by hepatic and extrahepatic tissue (13, 42), although some is excreted in urine (11, 17, 18, 32, 44, 63). Serum L-lactate clearance was not exponential. Previous studies show that serum L-lactate is cleared through two independent processes: hepatic removal follows second-order kinetics, whereas extrasplanchnic removal is linearly related to serum concentration (42).

In conclusion, D-lactate is a potent neurotoxic agent. In acidemic calves, the majority of neurological disturbances (i.e., ataxia and depressed menace, palpebral, and tactile reflexes) are related to D-lactate accumulation in CSF rather than in blood or to acidosis in CSF. However, HCl infusion has mild depressive effects on neurological function, and the suck reflex was similarly depressed by both DL-LA and HCl infusions. D-Lactate accumulation may be responsible for the majority of clinical signs of depression and loss of function in a variety of diseases, including neonatal calf diarrhea and other animal and human syndromes characterized by D-lactic acidosis. Research
needs to be undertaken to determine the precise mechanism of neurological depression caused by d-lactate.

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