Measurement of lactate in cerebrospinal fluid in investigation of inherited metabolic disease

Andrew Hutchesson,* Mary Anne Preece, George Gray, and Anne Green

Measurement of lactate concentrations in cerebrospinal fluid (CSF) has been suggested as part of the investigation of inborn errors of the electron transport chain, but little information exists regarding the reference range in children or the relationship between CSF and plasma concentrations. In 39 children without bacterial meningitis, diabetes, or recent seizures, we determined that the median (range) lactate concentrations in CSF and plasma collected concurrently were 1.4 (0.8–2.2) and 1.5 (0.6–2.3) mmol/L; the regression equation was CSF lactate = (0.38 ± 0.06) plasma lactate + 0.83 (r² = 0.14). In 8 of 11 (73%) children with electron transport chain defects, CSF lactate was ≥3.0 mmol/L; however, 2 of these 8 had a normal plasma lactate concentration. CSF lactate was also increased in 2 children with nonketotic hyperglycinemia. The finding that CSF lactate concentrations may be increased despite a normal plasma lactate value in children with electron transport chain defects is an important clue to the diagnosis of these disorders.

INDEXING TERMS: heritable disorders • pediatric chemistry • electron transport chain defects • hyperglycinemia • mitochondria

Lactic acidosis is a common consequence of tissue hypoxia and hypoperfusion, liver and renal failure, drug toxicity, and certain inborn errors of metabolism. This last group includes disorders of gluconeogenesis, the pyruvate dehydrogenase complex, the Krebs cycle, and the mitochondrial electron transport chain [1]. High concentrations of lactate in cerebrospinal fluid (CSF), as compared with that seen in controls, have been described in patients with bacterial, tuberculous, and fungal meningitis [2-4]; herpes simplex encephalitis [5]; status epilepticus [6]; and cerebral hypoxia and ischemia [7, 8]. High concentrations in CSF compared with concentrations in blood have also been demonstrated in several inborn errors of metabolism affecting the central nervous system (CNS), e.g., pyruvate dehydrogenase deficiency [9], mitochondrial myopathies [10, 11], and biotinidase deficiency [12]; measurement of lactate in CSF has also been advocated for investigating children with unexplained neurological disease [1, 13].

Despite this, little information is available about the reference interval for lactate in CSF in children. In addition, although CSF and blood lactate concentrations are thought to be independent, little evidence from humans is available to support this [5]. Here we report lactate concentrations in paired samples of CSF and venous plasma from patients with confirmed metabolic diseases affecting the CNS, and from control patients investigated for neurological symptoms (those with conditions known to affect CSF lactate being excluded).

Materials and Methods

Patients and controls. All children who were investigated for neurological disease between June 1993 and December 1995 at Children’s Hospital, Birmingham, UK, and from whom plasma and CSF samples collected concurrently were available for measurement of lactate were considered for the study. Controls were drawn from children who presented acutely with headache or meningeal irritation and who had a lumbar puncture performed for investigation of suspected meningitis. Exclusion criteria for the controls were a history of recent seizures; uncontrolled diabetes; evidence of intracranial hemorrhage; a final diagnosis of bacterial, tuberculous, or fungal meningitis [3]; or a plasma lactate concentration outside the reference interval (0.6–2.4 mmol/L). The patient group comprised all children with confirmed (on enzymatic or molecular biology grounds) inborn errors of metabolism affecting the CNS.

Specimen collection and analysis. CSF was obtained by lumbar puncture under sterile conditions, and venous blood was collected by venipuncture at the same time. All samples were collected into fluoride oxalate. Blood and...
any blood-stained CSF samples were centrifuged promptly. Analyses were performed within 30 min whenever possible (usually within 15 min); otherwise, plasma and supernatant CSF were stored at 4 °C for no longer than 3 days. Lactate was assayed with an Ektachem 700XR by means of lactate oxidase methodology (Johnson & Johnson, Rochester, NY). Use of this method for CSF had previously been validated in-house (unpublished) by comparison with an enzymatic method (Boehringer Mannheim UK, Lewes, UK). The between-batch CV for this assay is 1.5% over the lactate range 1–5 mmol/L.

Specimen collection (including sample volume) was dictated solely by clinical considerations, and no additional specimens were collected for the purposes of this study. In the patient group and in about half of the control group, CSF and plasma lactate assays had been requested as part of the clinical investigation, and the results were reported via routine channels. In the rest of the control group, CSF and plasma lactate assay had not been requested by clinicians. In those instances, we assayed lactate in the residual CSF and plasma after the results of all requested investigations had been reported. Lactate results in these children were not routinely made available to clinicians and did not influence patient management.

**Results**

The patient group comprised 13 children with inborn errors of metabolism (Table 1): 11 with mitochondrial disorders confirmed by analysis of respiratory chain enzyme activities in muscle biopsies [14, 15] or PCR analysis of mitochondrial DNA extracted from peripheral blood lymphocytes [16], and 2 with nonketotic hyperglycinemia (NKH) confirmed by assay of glycine cleavage enzyme activity in liver [17]. We also studied 39 control patients, ages 0.03–17.7 years (median 1.39 years).

The lactate concentrations in plasma and CSF from the control patients are shown in Fig. 1. The range of CSF lactate concentrations in this group was 0.8–2.2 mmol/L (median 1.4, mean 1.41, SD 0.28 mmol/L); the range of concentrations in plasma was 0.6–2.3 (median 1.5, mean 1.53, SD 0.43 mmol/L). Any differences seen between patients in the upper and lower halves of the age distribution were not significant, neither for CSF lactate (mean ± SD 1.49 ± 0.26 vs 1.37 ± 0.31 mmol/L, respectively; *P* >0.1) nor for plasma lactate (1.48 ± 0.24 vs 1.60 ± 0.49 mmol/L).

### Table 1. Details of patients with confirmed inborn errors of metabolism.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, years</th>
<th>Clinical features</th>
<th>Diagnostic criteria</th>
<th>Lactate conc, mmol/L&lt;sup&gt;*&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CSF</td>
</tr>
<tr>
<td><strong>Electron transport chain defects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.4</td>
<td>Liver failure, encephalopathy</td>
<td>Glutamate cytochrome reductase</td>
<td>0.04</td>
</tr>
<tr>
<td>2</td>
<td>0.1</td>
<td>Apneic episodes</td>
<td>Glutamate cytochrome reductase</td>
<td>0.02</td>
</tr>
<tr>
<td>3</td>
<td>1.2</td>
<td>Failure to thrive, hypotonia, nystagmus</td>
<td>Glutamate cytochrome reductase</td>
<td>0.019</td>
</tr>
<tr>
<td>4</td>
<td>6.5</td>
<td>Seizures, developmental delay</td>
<td>Succinate dehydrogenase</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>0.1</td>
<td>Hypertonia</td>
<td>Cytochrome c oxidase</td>
<td>0.026</td>
</tr>
<tr>
<td>6</td>
<td>1.1</td>
<td>Developmental delay, hypotonia, hepatomegaly</td>
<td>Cytochrome c oxidase</td>
<td>0.01</td>
</tr>
<tr>
<td>7</td>
<td>0.03</td>
<td>Hypotonia</td>
<td>Cytochrome c oxidase</td>
<td>0.03</td>
</tr>
<tr>
<td>8</td>
<td>1.2</td>
<td>Nystagmus, hypotonia, stupor</td>
<td>Cytochrome c oxidase</td>
<td>0.016</td>
</tr>
<tr>
<td>9</td>
<td>17.5</td>
<td>Ptosis, poor growth, fatigue</td>
<td>MELAS (3243A → G mutation)</td>
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</tr>
<tr>
<td>10</td>
<td>0.3</td>
<td>Hypotonia, developmental delay</td>
<td>NARP (8993 mutation)</td>
<td>5.8*</td>
</tr>
<tr>
<td>11</td>
<td>0.7</td>
<td>Cyanotic episodes</td>
<td>NARP (8993 mutation)</td>
<td>5.1*</td>
</tr>
<tr>
<td><strong>Nonketotic hyperglycinemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0.03</td>
<td>Seizures&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Glycine cleavage enzyme</td>
<td>1.4*</td>
</tr>
<tr>
<td>13</td>
<td>0.03</td>
<td>Seizures&lt;sup&gt;d&lt;/sup&gt;, hypotonia</td>
<td>Glycine cleavage enzyme</td>
<td>&lt;0.1*</td>
</tr>
</tbody>
</table>

<sup>a</sup> Enzyme activities are in μmol/min per gram wet weight, unless otherwise stated. Enzyme activities and molecular biology results are shown only when abnormal. Reference intervals for enzyme activities are glutamate cytochrome reductase, 0.10–1.00; pyruvate cytochrome reductase, 0.10–1.50; 2-oxoglutarate reductase, 0.10–1.00; succinate dehydrogenase, >25 000; cytochrome c oxidase, 0.10–5.00; glycine cleavage enzyme, 30–100 (nkat/kg).

<sup>b</sup> Abnormal lactate concentrations are indicated by asterisks.

<sup>c</sup> Ongoing.

<sup>d</sup> None for 3 days.

<sup>e</sup> nkat/kg.

MELAS, syndrome of mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes; NARP, syndrome of neurogenic weakness, ataxia, and retinitis pigmentosa.
mmol/L, respectively; P > 0.1). The correlation coefficient between CSF and plasma lactate concentrations in controls was 0.38 (P < 0.05).

Plasma lactate concentrations were >2.4 mmol/L in 8 of the 11 patients with confirmed mitochondrial disease but were normal in the remaining 3 patients (Table 1). Two of these three children had an abnormally high CSF lactate concentration, which had prompted further investigations leading to the diagnosis of defects in the electron transport chain. The sensitivity of an above-normal plasma lactate concentration (>2.4 mmol/L, the upper limit of the reference interval) for detecting mitochondrial disease was thus 73%. However, in clinical practice, minor increases in plasma lactate might be dismissed as insignificant. If we used a threshold of 3.0 mmol/L (reflecting local clinical practice), the sensitivity decreased to 64%.

The sensitivity of a CSF lactate concentration >2.2 mmol/L was also 73%, but this value was unchanged by increasing the threshold to 3.0 mmol/L. When the information from both investigations was combined, we determined that the sensitivity of an above-normal result in at least one test increased to 91%.

One of the patients with NKH, who had ongoing seizures (patient 8), had a grossly elevated value for plasma lactate (even greater than the concentration of lactate in CSF: 8.1 vs 5.8 mmol/L). The other (who showed no evidence of seizure activity for 3 days before specimen collection) had a normal plasma lactate concentration (2.3 mmol/L) and a CSF lactate of 3.5 mmol/L.

The ratio between CSF and plasma lactate concentrations varied among the control patients, being highest in those with a low plasma lactate. However, the ratio of CSF:plasma lactate concentration did not provide any diagnostic information over that from the absolute concentration in CSF—the ratios in patients (0.58–1.76, median 0.98) being similar to those seen in controls (range 0.60–1.67, median 0.97).

**Discussion**

Measurement of plasma lactate is frequently used as a first-line test to assess children thought to have inborn errors affecting the respiratory chain, although an increased concentration is not characteristic of all mitochondrial disorders (e.g., Leber hereditary optic atrophy). Our results show that lactate concentrations may be increased to a greater extent in CSF than in plasma; furthermore, they may be substantially increased in CSF in the presence of a normal or only slightly increased plasma concentration. This could reflect the heteroplasmy of mitochondrial defects (the random variation in the proportion of normal and defective mitochondria) in different tissues, but the similar findings found by us in patients with NKH (in the absence of recent seizures) and by others in patients with X-linked pyruvate dehydrogenase deficiency [9] suggest that the increase in CSF lactate may result from tissue specificity of electron transport chain proteins. Alternatively, increased lactate in CSF could result from the high energy demand and lactate production of the CNS (which accounts for 2% of body weight but requires ~13% of the resting cardiac output) [11, 18].

Three children, two with cytochrome c oxidase deficiency and one with succinate dehydrogenase deficiency, had a normal CSF lactate concentration. Tissue-specific isoforms of both of these enzymes are thought to exist [19, 20]; accordingly, in some patients, these defects may show greater expression in liver or muscle than in the CNS. In addition, intractable seizures in child 4 (Table 1) may have led to an increased plasma lactate concentration.

The range of CSF lactate concentrations we observed in controls is lower than reported previously [3, 5, 6, 21]. Although the association between lactate concentrations in CSF and plasma is significant, this accounts for only 13% of interindividual variability in CSF lactate concentrations. Because analytical variability would account for only a further 1.5% variation in each sample type, lactate concentrations in CSF appear to be largely independent of plasma concentrations within the reference interval. The slope of the regression line obtained indicates that lactate concentrations show less interindividual variability in CSF than in plasma. These results agree with those of Posner and Plum [5], who showed that lactate concentrations in CSF could alter independently of those in plasma, and could remain stable for several days despite pharmacological alteration of plasma pH. Clearance of lactate from the CSF, especially from that surrounding the cauda equina, is likely to be slower than from blood. Also, because it is strongly ionized at physiological pH (pK, 3.08) [22], lactate is unlikely to cross the blood–brain...
barrier from plasma; lactate concentrations in CSF should therefore reflect production within the CNS.

Investigations for defects in the electron transport chain and the pyruvate dehydrogenase complex (such as assay of electron transport chain function in muscle and pyruvate dehydrogenase activity in skin fibroblasts, and molecular biology studies of mitochondrial DNA) are invasive, time-consuming, and expensive. Although CSF lactate was not increased in all children with metabolic disease affecting the CNS in this study, the finding of a normal CSF lactate concentration may increase the threshold for performing these procedures. Conversely, a high CSF lactate concentration requires an explanation; in the absence of seizures, systemic metabolic disease, and intracranial infection, inborn errors of metabolism are an important cause. We have found CSF lactate concentration to be a useful tool in assessing children with suspected inborn errors who present with neurological disease, even when the lactate concentration in plasma is within the reference interval.

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