TRANSPORT OF 5-METHYLTETRAHYDROFOLIC ACID INTO THE CEREBROSPINAL FLUID IN MAN

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SUMMARY

1. 5-Methyltetrahydrofolate appeared in the CSF when given intravenously to man.
2. The data suggest considerable circulation of folate between CSF and other body compartments.

Key words: cerebrospinal fluid, methyltetrahydrofolic acid.

Folic acid is one of the few substances known to be present in higher concentration in the cerebrospinal fluid (CSF) than in other body fluids. CSF folate levels are approximately three times higher than serum levels (Herbert & Zalusky, 1961) and the relationship between serum and CSF folate remains constant in the presence of folate deficiency (Reynolds, Gallacher, Mattson, Bowers & Johnson, 1970). However, the administration of pharmacological doses of folic acid to folate-deficient epileptic patients for 3 months had no effect on CSF folate levels despite a considerable elevation of serum folate, suggesting the presence of a considerable blood–brain barrier mechanism for this vitamin (Spaans, 1970). Shaw, MacSweeney, Johnson, O'Keeffe, Naidoo, MacLeod, Jog, Preece & Crowley (1971) reported a very slow rise in CSF folate levels after 6 months' treatment with the vitamin in demented (non-epileptic) patients. The concept of a blood–brain barrier mechanism is also supported by the experimental studies of Obbens (1973) and Hommes, Obbens & Wiffels (1973). Alperin & Haggard (1970) reported a rise in CSF folate 3 days after an injection of 34 μmol (15 mg) of folic acid or 25.4 μmol (12 mg) of folinic acid. There was no rise in CSF folate after 2 weeks on 0.227 μmol (100 μg) of oral folate daily but a gradual rise on 1.14 μmol (500 μg) of folate daily.

As part of a study of the interrelations of folic acid and vitamin B₁₂ with respect to the nervous system we have examined the uptake of folic acid into the CSF in man. The form of folate normally present in serum and CSF is only detectable microbiologically with *Lactobacillus casei* and is almost certainly 5-methyltetrahydropteroylglutamic acid. For this reason we have measured the appearance in the CSF of tritium-labelled 5-methyltetrahydropteroylglutamic acid which was given to these patients by parenteral injection.

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MATERIALS AND METHODS

Preparation of $^3$H-labelled 5-methyltetrahydrofolate

1-06 mmol (500 mg) of 5-formyltetrahydrofolic acid was labelled generally with tritium by heating in tritiated water in the presence of a platinum catalyst (The Radiochemical Centre, Amersham). The tritiated compound was purified by column chromatography (Nixon & Bertino, 1971), and used for the synthesis of 5-methyltetrahydrofolic acid by the method of Chanarin & Perry (1967). The solution of tritiated 5-methyltetrahydrofolic acid was sterilized by passage through a Millipore Millex disposable filter unit (0-22 μm). A portion was retained for use as the dose standard.

Procedure

A freshly prepared solution of 5-$^3$Hmethyltetrahydrofolic acid was given intravenously at a dose of 10-8 nmol (5 μg), as the L form, per kg of body weight. Tritium activity was of the order of 50 μCi, the volume injected being about 2 ml.

Observations were made on twelve patients, all of whom required a lumbar puncture carried out as a diagnostic procedure. All the patients had evidence of neurological disease. Six had peripheral neuropathy, two epilepsy, one temporal arteritis, two alcoholism and one pernicious anaemia. All the patients gave informed consent to a single injection of labelled folate being given between 4 and 24 h before the lumbar puncture. The study was approved by the hospital ethical committee.

Microbiological assay methods

Specimens were assayed with Lactobacillus casei used as the test organism. Dehydrated culture medium (B.B.L.) was used. Samples were heated at 116°C for 10 min after 1:50 dilution in 0.1 mol/l phosphate buffer containing 0.15 g of ascorbate/100 ml. Some CSF specimens were also assayed in untreated samples without heating by using a chloramphenicol-resistant strain of L. casei in the presence of chloramphenicol (Chanarin, Kyle & Stacey, 1972). There was no significant difference in folate values obtained with either method.

Measurement of tritium activity

CSF (1 ml) was added to 4 ml of NCS solubilizer (Amersham/Searle Corporation) and shaken until the solution cleared. Liquid scintillator (10 ml of 2,5-diphenyloxazole, 6 g/1 of toluene) was added and the tritium activity counted in an LKB-Wallac liquid-scintillation counter.

The dose standard (10 μl) was added to a second portion (1 ml) of CSF and prepared for counting in the same way. The concentration of radioactive folate in the test samples was determined from the ratio of sample radioactivity counts to standard counts, and results were expressed as radioactive folate in nmol/l of CSF.

RESULTS

The results (Table 1 and Fig. 1) show that after an injection of labelled folate a significant amount of the dose appeared in the CSF. The highest proportion of the parenteral dose was found 4–5 h after the injection and lower levels 20–24 h later.

The plasma folate level was lower than that found in CSF but the radioactivity was higher in plasma than that in CSF.
TABLE 1. Total and labelled folate in serum and CSF after intravenous administration of $^3$H-labelled 5-methyltetrahydrofolate. $5-[^3$H]$\text{Methyltetrahydrofolate (5~\mu g/kg)}$ was injected intravenously 4–24 h before a lumbar puncture (LP). The time-lag between injection and lumbar puncture (column 1), dose (column 2), concentration and radioactivity of folate in the CSF (columns 3, 4 and 5) and serum folate (columns 6 and 7) are shown.

<table>
<thead>
<tr>
<th>Time elapsing between i.v. injection and lumbar puncture (h)</th>
<th>Dose (i.v.) [\mu mol (\mu g)]</th>
<th>Total folate in CSF [nmol/l (ng/ml)]</th>
<th>Labelled folate in CSF [nmol/l (ng/ml)]</th>
<th>% Labelled CSF</th>
<th>Total serum folate at time of LP [nmol/l (ng/ml)]</th>
<th>Labelled folate in serum [nmol/l (ng/ml)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.63 (290)</td>
<td>48 (22)</td>
<td>6.7 (3.5)</td>
<td>16</td>
<td>17 (8.0)</td>
<td>20 (9.0)</td>
</tr>
<tr>
<td>$4\frac{1}{2}$</td>
<td>0.58 (265)</td>
<td>22 (10)</td>
<td>3.9 (1.8)</td>
<td>18</td>
<td>17 (8.0)</td>
<td>5.2 (2.4)</td>
</tr>
<tr>
<td>5</td>
<td>0.68 (315)</td>
<td>81 (37.5)</td>
<td>22 (10)</td>
<td>27</td>
<td>46 (21)</td>
<td>20 (9.0)</td>
</tr>
<tr>
<td>7</td>
<td>0.52 (240)</td>
<td>40 (18.5)</td>
<td>3.3 (1.5)</td>
<td>8</td>
<td>19 (8.5)</td>
<td>6.6 (3.0)</td>
</tr>
<tr>
<td>20</td>
<td>0.72 (330)</td>
<td>35 (16)</td>
<td>3.5 (1.65)</td>
<td>10</td>
<td>19 (8.5)</td>
<td>7.2 (3.3)</td>
</tr>
<tr>
<td>21</td>
<td>0.66 (305)</td>
<td>39 (18)</td>
<td>3.9 (1.82)</td>
<td>10</td>
<td>14 (6.5)</td>
<td>9.6 (4.4)</td>
</tr>
<tr>
<td>22</td>
<td>0.81 (370)</td>
<td>46 (21)</td>
<td>2.8 (1.31)</td>
<td>6</td>
<td>10 (4.5)</td>
<td>5.7 (2.6)</td>
</tr>
<tr>
<td>23</td>
<td>0.58 (265)</td>
<td>47 (21.4)</td>
<td>3.4 (1.6)</td>
<td>7.5</td>
<td>22 (10)</td>
<td>8.0 (3.7)</td>
</tr>
<tr>
<td>23</td>
<td>0.73 (335)</td>
<td>54 (25)</td>
<td>3.3 (1.5)</td>
<td>6</td>
<td>10 (4.5)</td>
<td>6.6 (3.0)</td>
</tr>
<tr>
<td>24</td>
<td>0.44 (200)</td>
<td>22 (10)</td>
<td>2.2 (1.0)</td>
<td>10</td>
<td>31 (14)</td>
<td>4.3 (2.0)</td>
</tr>
<tr>
<td>24</td>
<td>0.52 (240)</td>
<td>37 (17)</td>
<td>3.3 (1.5)</td>
<td>8.8</td>
<td>13 (6.0)</td>
<td>10 (4.5)</td>
</tr>
<tr>
<td>24</td>
<td>0.53 (255)</td>
<td>54 (25)</td>
<td>5.4 (2.5)</td>
<td>10</td>
<td>37 (17)</td>
<td>18 (8.2)</td>
</tr>
</tbody>
</table>

FIG. 1. Labelled folate in CSF after an intravenous injection of 10.8 nmol/kg (5 \mu g/kg) of $5-[^3$H]-methyltetrahydrofolate given 4–24 h before the lumbar puncture.
Although CSF folate levels are not readily changed by administration of folic acid (Spaans, 1970; Shaw et al., 1971), our results show that there is nevertheless a considerable circulation of methylenetetrahydrofolate between plasma and CSF. Up to one-fifth of the CSF folate at 4 h after a 648 nmol (300 µg) dose is derived from the plasma compartment. This is consistent with the observations of Levitt, Nixon, Pincus & Bertino (1971) on CSF folate transport in dogs. The relative failure of CSF folate levels to rise during prolonged treatment despite the demonstrable entry of a labelled dose of the vitamin suggests either a rapid uptake of the labelled folate into brain tissue or further distribution to other body fluids and tissues. The lower proportion of labelled vitamin at 24 h than at 4 h (Fig. 1) is consistent with either interpretation.

There is an increasing interest in mechanisms of transport of folate within the body. Thus transport of folate analogues across the gut wall is accompanied by conversion of these analogues into 5-methyltetrahydropteroylglutamic acid (Chanarin & Perry, 1969; Perry & Chanarin, 1973). Uptake of folate by the liver leads to its conversion into formyl- and methylpolyglutamates (Shin, Williams & Stokstad, 1972; Corrocher, Bhuyan & Hoffbrand, 1972; Houlihan & Scott, 1972). Transport of 5-methyltetrahydrofolate into cells is vitamin $B_{12}$-dependent (Das & Hoffbrand, 1970; Tisman & Herbert, 1973), and this is the probable explanation for the low red cell folate level encountered in untreated pernicious anaemia (Hansen, 1964; Cooper & Lowenstein, 1964). In this study it has been shown that there is rapid transport of 5-methyltetrahydrofolate between plasma and CSF in man. The extent to which this transport is influenced by vitamin $B_{12}$ or folate deficiency and by anticonvulsant drugs is being studied.

ACKNOWLEDGMENT

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REFERENCES


