Transfer of Magnesium across the Perfused Choroid Plexus of Sheep

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Abstract

Isolated perfused choroid plexus preparations from sheep were used to study the effects of low concentrations of magnesium in the perfusion fluid on the transfer of magnesium into choroid plexus fluid (CPF). A perfusion fluid of similar electrolyte composition to sheep blood resulted in CPF similar to ventricular cerebrospinal fluid at a rate of 2.2 μl min⁻¹ mg⁻¹ dry choroidal tissue. Decreasing the concentration of magnesium in the perfusion fluid caused a fall in the concentration of magnesium in the CPF, although it remained higher than in the perfusion fluid. The rate of transfer of magnesium from the perfusion fluid to the CPF decreased in the presence of high levels of potassium in the perfusion fluid. But decreasing the concentration of calcium in the perfusion fluid had no effect on magnesium transfer rates.

These results suggest that the ability of the choroid plexus to transport magnesium against a concentration gradient is an important control of the concentration of the cerebrospinal fluid. However, this ability is insufficient to maintain cerebrospinal fluid concentrations of magnesium at normal levels when the blood magnesium concentration is below about 0.5 mmol l⁻¹.

Extra keyword: hypomagnesaemia.

Introduction

The magnesium concentration of cerebrospinal fluid (CSF) usually remains constant even when the concentration of magnesium in plasma is varied (Oppelt et al. 1963; Reed and Yen 1978). However, protracted and severe hypomagnesaemia will cause a decrease in the concentration of magnesium in the CSF of both sheep (Meyer and Scholz 1972) and cattle (Pauli and Allsop 1974). An examination of the relationship between concentrations of magnesium in blood and ventricular CSF in cattle (Allsop and Pauli 1985) indicated that once the blood magnesium concentration had fallen below 0.5 mmol l⁻¹ the magnesium concentration of the CSF began to decline until, after various periods of time, it had fallen to about 0.5 mmol m⁻¹ when tetany supervened. Clinical signs of tetany in sheep have also been produced by perfusion of the CSF space with an artificial CSF solution low in magnesium (Allsop and Pauli 1975). However, instances of grazing cattle with severe hypomagnesaemia but normal concentrations of magnesium in lumbar CSF, and no tetany (Pauli and Allsop 1974) may indicate that factors other than the magnesium concentration of blood affect the transport of magnesium into the CSF. Parkinson and Leaver (1980) demonstrated that experimental hyperkalaemia lowered the concentration of magnesium in sheep CSF. There is also some evidence that a decrease in blood calcium concentrations of hypomagnesaemic animals may precipitate tetany (Hemingway and Ritchie 1965), although the effect was not found consistently (Scholz and Meyer 1973).
To clarify the relationship between blood and CSF levels of magnesium and to investigate some of the factors that may be responsible for modifying the transport of magnesium to the CSF, a system for perfusing the isolated choroid plexus of sheep was used. This system enabled direct measurement of the rate of synthesis and composition of fluid from the choroid plexus to be made in response to changes in the composition of the perfusion fluid (Segal and Pollay 1977).

Materials and Methods

Perfusion Solutions

The perfusion solutions were 30% (v/v) suspensions of fresh, saline-washed, ovine erythrocytes in Krebs–Henseleit bicarbonate buffer, pH 7·4, modified as follows. The reference solution which was perfused once into each choroid plexus preparation contained (per litre) 4·6 mmol K+; 150 mmol Na+; 2·35 mmol Ca2+; 0·81 mmol Mg2+ and 1·5 mmol inorganic phosphate. The other eight perfusates used were obtained by altering the magnesium concentration and/or the calcium and potassium concentrations (Tables 2 and 3) with the ionic strength being kept constant by minor alterations in the concentration of sodium chloride. All perfusates contained 70 g l⁻¹ of ovine serum albumin (A3264, Sigma Chemical Co.) and 0·6 mmol l⁻¹ glucose.

Perfusion System

Twelve Romney-cross ewe hoggets weighing 30–35 kg were used. The procedure for the perfusion of the choroid plexus was based on that of Pollay et al. (1972). Each sheep was killed without prior anaesthesia by transverse incision of the ventral neck region, which severed all the soft tissues including the major blood vessels, followed immediately by dislocation of the neck and severance of the spinal cord at the occipito-atlantal junction. This was carried out rapidly by a proficient slaughterman. Its head was removed and infused with cold (4°C) Krebs–Henseleit bicarbonate buffer, pH 7·4, introduced through the carotid arteries. The skull was skinned, the cranial bones removed and the brain lifted out with care being taken to preserve the ventral blood vessels intact. Cannulae (vinyl tubing, Dural Plastics, Australia) were sealed into one internal carotid artery (0·75 mm i.d. tubing) and into the cerebral vein (1·2 mm i.d. tubing) with tissue glue (COAPT, Gewebekleber, Ethicon GmbH, Hamburg) and secured with ligatures. Superficially visible branches of the anterior carotid artery were ligated. No attempt was made to free the choroid plexus and its blood supply from the surrounding tissue. As soon as the cannulae were in place the perfusion was begun; this was always within 30 min of the death of the sheep. The perfusions were carried out in a room at 37°C.

The perfusion solutions were pumped through a multi-bulb oxygenator (Hems et al. 1966) through which was passed 5% (v/v) carbon dioxide in oxygen. The free surface of the fluid reservoir was 1·2–1·3 m above the tissue to give an inflow pressure of about 12–13 kPa. The solutions were passed through an inline filter (McGaw Ethicals, Auckland, New Zealand). The inflow pressure was adjusted to maintain a relatively constant venous outflow rate.

The dorsal surface of the brain was cut and reflected to expose the perfused choroid plexus within the lateral ventricle. The foramen of Munro was sealed with tissue glue and the chamber of the ventricle filled with light white oil (M3516, Sigma Chemical Co.). Droplets of fluid emerging from the choroid plexus and accumulating under the oil were collected at intervals in glass micropipettes as described by McFarland and Reed (1972).

The criteria used to evaluate the functional state of the tissue were the inflow pressure required to maintain a venous outflow of 250–300 μl min⁻¹ and a rate of production of clear choroid plexus fluid (CPF) of at least 20 μl min⁻¹. Evans blue dye was added to the perfusion solution at the conclusion of some experiments to verify that the dye did not enter the CPF. Each preparation was perfused for about 3 h during which time three or four solutions of different electrolyte composition were used. Each solution was perfused for 45 min, the first 15 min being used for equilibration and the rest for collection of CPF and perfusate. The order in which the different perfusion solutions were used was selected randomly for each preparation to avoid systematic carry-over effects.

At the conclusion of perfusion of each preparation, the choroidal tissue was removed, blotted and weighed. It was reweighed after drying overnight at 100°C.
Analyses

Microhaematocrits of the perfusion fluid, both before and after passage through the tissue, were measured at 15-min intervals. The rates of collection of perfusate and CPF were measured at 5-min intervals. Concentrations of sodium and potassium were measured by flame photometry and of magnesium and calcium by atomic absorption spectrophotometry. The method of Lowry et al. (1951) was used to measure protein concentrations.

Statistical Analyses

The parameters of CPF production and magnesium transfer in different perfused preparations were compared after log transformation followed by analysis of variance and Neuman–Keul's multiple-range test.

Results

Rate of Venous Outflow

Once the flow of perfusate had been established, the rate of outflow of perfusion solution was generally stable for at least 3 h. A mean value (±s.e.m.) of 268 ± 4 µl min⁻¹, which was equivalent to 17·2 ± 0·8 µl min⁻¹ mg⁻¹ dry matter, was obtained from the 12 preparations. After about 3 h the flow rate often decreased and experimental collections were discontinued. The alterations of the electrolyte composition of the perfusion fluid used in these experiments had no effect on the flow rate of perfusate.

Evans blue dye was shown microscopically to enter the stroma of the choroid plexus but did not appear in the CPF.

Table 1. Composition of ventricular cerebrospinal fluid before perfusion and of choroid plexus fluid formed during perfusion of the reference solution

Values are means (±s.e.m.) from 12 sheep. Concentrations are expressed as mmol l⁻¹ except for protein which is given as g l⁻¹

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Ventricular CSF</th>
<th>Choroid plexus fluid</th>
<th>Constituent</th>
<th>Ventricular CSF</th>
<th>Choroid plexus fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>157·4 ± 5·1</td>
<td>154·4 ± 3·2</td>
<td>Phosphorus</td>
<td>0·52 ± 0·01</td>
<td>0·50 ± 0·01</td>
</tr>
<tr>
<td>Potassium</td>
<td>2·83 ± 0·03</td>
<td>3·22 ± 0·06</td>
<td>(inorganic)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>1·12 ± 0·03</td>
<td>1·17 ± 0·02</td>
<td>Glucose</td>
<td>2·50 ± 0·26</td>
<td>2·64 ± 0·23</td>
</tr>
<tr>
<td>Magnesium</td>
<td>1·07 ± 0·08</td>
<td>1·04 ± 0·07</td>
<td>Protein</td>
<td>0·43 ± 0·06</td>
<td>0·52 ± 0·07</td>
</tr>
</tbody>
</table>

Rate of Synthesis and Composition of Choroid Plexus Fluid

When perfused with reference solution, the mean rate of synthesis (±s.e.m.) of CPF by the 12 preparations was 2·18 ± 0·10 µl min⁻¹ mg⁻¹ dry choroidal tissue (mean dry weight = 15·6 ± 0·6 mg; 16% dry matter) when measured by direct collection of fluid from the choroid plexus and 1·81 ± 0·13 µl min⁻¹ mg⁻¹ dry tissue when calculated from the difference in haematocrit values of the arterial and venous perfusion solutions (Pollay et al. 1972). Although these values are significantly different (P < 0·01; paired t-test) they are significantly correlated (r = 0·68; P < 0·05). Estimates of the rate of transfer of magnesium into the CPF are all based on the rate of CPF formation obtained by direct collection of fluid.

The electrolyte composition of the CPF collected during perfusion of the reference solution is given in Table 1, together with the composition of native CSF collected from the lateral ventricles of the brain before perfusion. The concentration of magnesium in the CPF was usually higher than that in the perfusion fluid,
whereas the relationship was reversed for calcium. The calculated mean (± s.e.m.) rate of transfer of magnesium from perfusate to CPF was 2.28 ± 0.20 nmol l⁻¹ min⁻¹ mg⁻¹ dry choroid.

**Effect of Altering the Concentration of Magnesium in the Perfusion Solution**

Changing the concentration of magnesium in the perfusion solution caused no change in the rate of formation of CPF. The relationship between the magnesium concentrations of the perfusion solutions and of CPF is shown in Fig. 1. A curve of the form:

\[ y = y_\infty [1 - \exp(-5.1x)] \]

was fitted to the mean data for each perfusion fluid magnesium concentration (x).

![Fig. 1](image)

**Fig. 1.** Magnesium concentrations of choroid plexus fluid in relation to those of perfusion fluid. Points are means ± s.e.m. Numbers in parentheses are the number of perfusions at each perfusion fluid magnesium concentration. The line is described by the equation \( y = 1.06 [1 - \exp(-5.1x)] \).

The magnesium concentration of the CPF (y) reaches an asymptotic value (\( y_\infty \)) of 1.06 mmol l⁻¹. Fitting this curve assumes that the rate of transfer of magnesium across the choroid plexus is approaching a maximum at a perfusion fluid magnesium concentration of 0.81 mmol l⁻¹. There is evidence to show that CSF magnesium concentrations do not increase rapidly when the concentration of magnesium in the blood is elevated above normal (>0.8 mmol l⁻¹) (Oppelt et al. 1963; Reed and Yen 1978). This model also assumes that the curve passes through the origin. At 0.01 mmol l⁻¹ magnesium in the perfusion fluid the measured concentrations of magnesium in CPF were close to the detection limit in the available sample and the values are not very reliable so this assumption is valid for the data available.
The curves derived for the individual brain preparations showed no consistent between-preparation effect so all the results have been treated as being independent for statistical analysis. There were no significant changes in any of the other measured constituents of the CPF as the concentration of magnesium was decreased.

The calculated mean rates of transfer of magnesium from the perfusion solution to the CPF are shown in Table 2. The rate of transfer was highly correlated \((r = 0.80; \ P < 0.001; \ n = 30)\) with the magnesium concentration of the perfusion solution. The kinetic parameters of the rate of transfer of magnesium \((v)\) in relation to the concentrations of magnesium the perfusion solutions \((s)\) were estimated by statistical evaluation of the Michaelis–Menten equation (Wilkinson 1961). These calculations gave \(K_m(\pm \text{s.e.m.}) = 0.29 \pm 0.23 \text{ mmol}^{-1}\) and \(V_{\text{max.}} = 3.1 \pm 1.7 \text{ nmol}^{-1} \text{ min}^{-1} \text{ mg}^{-1} \text{ dry choroid}\).

### Table 2. Rate of transfer of magnesium into choroid plexus fluid (CPF) in relation to the magnesium concentration of the perfusion solution

<table>
<thead>
<tr>
<th>Conc of magnesium in perfusion solution (mmol 1(^{-1}))</th>
<th>Mean rate (± s.e.m.) of CPF production (μl min(^{-1}) mg(^{-1}) dry matter)</th>
<th>Rate of transfer of magnesium into CPF (mmol 1(^{-1}) min(^{-1}) mg(^{-1}) dry matter)(^A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.81</td>
<td>12</td>
<td>2.18 ± 0.10</td>
</tr>
<tr>
<td>0.52</td>
<td>5</td>
<td>1.78 ± 0.18</td>
</tr>
<tr>
<td>0.31</td>
<td>5</td>
<td>1.69 ± 0.17</td>
</tr>
<tr>
<td>0.11</td>
<td>5</td>
<td>1.99 ± 0.12</td>
</tr>
<tr>
<td>0.01</td>
<td>3</td>
<td>1.80 ± 0.12</td>
</tr>
</tbody>
</table>

\(^A\) Geometric mean. Values in parentheses are ranges of ±1 standard deviation. Values with no letter in common are significantly different \((P < 0.01)\), analysis of variance followed by Neuman–Keul’s multiple-range test on log transformed data.

### Table 3. Effect of varying calcium, potassium and magnesium concentrations in the perfusion fluid on the transfer of magnesium across the choroid plexus

<table>
<thead>
<tr>
<th>Perfusion fluid concentrations (mmol 1(^{-1}))</th>
<th>Mean rate (± s.e.m.) of CPF production (μl min(^{-1}) mg(^{-1}) dry matter)</th>
<th>Mean concn (± s.e.m.) of magnesium in CPF (mmol 1(^{-1}))</th>
<th>Rate of magnesium transfer (mmol 1(^{-1}) min(^{-1}) mg(^{-1}) dry matter)(^A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg(^{2+}) Ca(^{2+}) K(^+)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.81 2.35 4.6</td>
<td>2.18 ± 0.10</td>
<td>1.04 ± 0.07</td>
<td>2.16 (1.98, 2.37)</td>
</tr>
<tr>
<td>0.81 1.50 4.6</td>
<td>1.98 ± 0.10</td>
<td>0.95 ± 0.11</td>
<td>1.84 (1.72, 1.96)</td>
</tr>
<tr>
<td>0.81 2.35 6.5</td>
<td>1.67 ± 0.06*</td>
<td>0.62 ± 0.06*</td>
<td>1.02 (0.96, 1.10)**</td>
</tr>
<tr>
<td>0.31 2.35 4.6</td>
<td>1.69 ± 0.17</td>
<td>0.76 ± 0.08</td>
<td>1.21 (1.03, 1.43)</td>
</tr>
<tr>
<td>0.31 1.50 4.6</td>
<td>1.75 ± 0.10</td>
<td>0.62 ± 0.06</td>
<td>1.06 (0.99, 1.13)</td>
</tr>
<tr>
<td>0.31 2.35 6.5</td>
<td>1.48 ± 0.06</td>
<td>0.33 ± 0.04*</td>
<td>0.49 (0.43, 0.57)**</td>
</tr>
</tbody>
</table>

\(^A\) Geometric means. Values in parentheses are ranges of ±1 standard deviation.
Effect of Lowering the Calcium Concentration of the Perfusion Fluid

Reducing the calcium concentration of the perfusion fluid from 2·35 to 1·5 mmol l⁻¹ had no significant effect on the rate of formation of CPF or on the magnesium concentrations of the CPF produced using perfusion fluids containing 0·81 or 0·31 mmol l⁻¹ magnesium. Consequently, the rates of transfer of magnesium from the perfusion fluid to the CPF were not significantly different (Table 3). The calcium concentration of the CPF formed from perfusion solutions with a concentration of 1·5 calcium (mean ± s.e.m. 1·10 ± 0·02) mmol l⁻¹ was not significantly different from that obtained using solutions of 2·35 calcium (mean 1·17 ± 0·02) mmol l⁻¹.

Effect of Increasing the Concentration of Potassium in the Perfusion Fluid

Raising the potassium concentration of the perfusion fluid from 4·6 to 6·5 mmol l⁻¹ at magnesium concentrations of 0·81 and 0·31 mmol l⁻¹ (Table 3) significantly decreased the concentration of magnesium in the CPF and tended to lower the rate of formation of CPF. As a result the calculated rate of transfer of magnesium across the choroid plexus decreased significantly. The mean (± s.e.m.) potassium concentration of the CPF (3·67 ± 0·10 mmol l⁻¹) formed from the high-potassium (6·5 mmol l⁻¹) perfusion fluid was significantly higher (3·21 ± 0·05 mmol l⁻¹; P < 0·001; t-test) than that from normal potassium (4·6 mmol l⁻¹) perfusion solution.

Discussion

The rate of flow of perfusion solution was similar to that obtained by Pollay et al. (1972). Pappenheimer and Setchell (1972) estimated the rate of blood flow through the brain of conscious sheep to be 1 µl min⁻¹ mg⁻¹ wet weight rising to 2 µl min⁻¹ mg⁻¹ when the sheep were anaesthetized with halothane. This compares with a mean rate of 2·7 µl min⁻¹ mg⁻¹ wet tissue in the present choroid plexus perfusions. The rate of vascular flow has been shown to affect the rate of synthesis and composition of CSF (Deane and Segal 1978, 1979) so great care was taken to obtain a similar flow of perfusion solution through each brain preparation. While there was still considerable variation in the rate of formation and composition of CPF, sufficient uniformity was obtained to detect significant effects due to changing the composition of the perfusion fluid.

It is not clear why the direct collection of CPF gave a higher value for the rate of synthesis than that calculated from the change in haematocrits. The perfusion fluid flow was probably not restricted to the choroid plexus tissue because no attempt was made to locate and block any deeper branches of the choroidal artery. It is possible, therefore, that the red-cell-free solution used initially to clear the brain of blood was being gradually displaced by perfusion solution, thus contributing to lower haematocrit values in the venous outflow solution. However, there was no significant change in the calculated rate of CPF production throughout the 3 h of each perfusion. The discussion here is based on the rate of CPF formation obtained by direct collection.

The rate of formation of CPF was greater than that reported for sheep by Pollay et al. (1972) but similar to that derived for goats by ventriculo-cisternal perfusion (Heisey et al. 1962). There is considerable variation in estimates for the rate of production of CSF per unit weight of choroidal tissue in various species (Pollay 1975),
but the mean value of 0.35 µl min⁻¹ mg⁻¹ wet tissue obtained in the present experiments is similar to that for some other species.

The composition of the CPF formed during perfusion of the reference solution was similar to native CSF and to ventricular CSF collected from conscious sheep (Beal and Bligh 1977). The observation that Evans blue dye did not cross the choroidal ependyma indicates that the structural integrity of the membrane was preserved. All these observations suggest that when a solution of similar composition to blood was perfused through the tissue, a CPF of similar quantity and composition to CSF was formed.

The shape of the curve relating perfusion fluid and CPF magnesium concentrations is similar to those described for the relationship between blood and CSF magnesium concentrations in sheep (Meyer and Scholz 1972) and cattle (Allsop and Pauli 1985) with the decrease in the CPF levels of magnesium becoming more pronounced when the perfusion fluid magnesium concentration fell below about 0.5 mmol l⁻¹. The equation used to represent these results takes account of the likelihood that the magnesium concentration of the CPF reaches an asymptote and that the curve passes through a true zero. The asymptotic value for the CPF magnesium concentration is similar to the magnesium concentration of ventricular CSF.

The rate of transfer of magnesium across the choroid plexus at different perfusion fluid magnesium concentrations followed a similar curve to that in Fig. 1. The estimated value for $K_m$ for the transport of magnesium from the perfusion fluid to the CPF (0.29 ± 0.23 mmol l⁻¹) indicates a transport system of high affinity that is saturated at a concentration of about 0.6 mmol l⁻¹. This is consistent with the in vivo findings mentioned above. The high affinity of the choroid plexus transport system suggests that it plays an important role in maintaining CSF magnesium concentrations in the initial stages of hypomagnesaemia, but that if circulating magnesium levels fall too low then the transport system can no longer provide sufficient magnesium to fulfil the requirements for production of normal CSF. Meyer (1977) claimed that CSF magnesium concentrations were primarily maintained by decreased outflow of magnesium rather than by transport at the choroid plexus. The results presented here show that the choroid is able to transport magnesium against a large concentration gradient and suggest that it plays a major role in the homeostatic control of the magnesium concentrations of the CSF in response to fluctuations in the blood magnesium levels.

Decreasing the calcium concentration of the perfusion fluid in the presence of normal or low concentrations of magnesium did not affect the rate of transfer of magnesium into the CPF. This suggests that the hypocalcaemia that often accompanies severe hypomagnesaemia is not a factor in the decline in CSF magnesium levels leading to tetany. Neither was there a significant decline in CPF calcium levels which may have aggravated the effects of reduced CPF magnesium concentrations. Allsop and Pauli (1975) showed that lowering the magnesium and calcium concentrations of the CSF by ventriculolumbar perfusion caused more severe clinical signs in sheep than those evoked by lowering the concentrations of each ion separately.

Increasing the concentration of potassium in the perfusion fluid had a significant effect on the rate of transfer of magnesium into the CPF at both normal and low magnesium concentrations in the perfusion fluid. Experimental hyperkalaemia has been shown to decrease the CSF magnesium concentrations of ventriculocisternally
perfused sheep (Parkinson and Leaver 1980). However, the role of blood potassium concentrations in clinical grass tetany is not clear and observations suggest that they begin to increase only after the onset of tetany (Pauli and Allsop 1974). Possibly high circulating potassium concentrations, by inhibiting magnesium transfer into the CSF, exacerbate the tetany or delay recovery following magnesium treatment. The increased concentrations of potassium in the CPF due to increased potassium in the perfusion fluid have been demonstrated previously (Pollay et al. 1973).

While these experiments have not resolved the question as to why some animals can apparently withstand the effects of hypomagnesaemia, they have clarified the relationship between the magnesium contents of blood and of CSF and they indicate that CSF magnesium levels may be maintained by other means in such animals. Two possibilities are extrachoroidal synthesis of a CSF of high magnesium concentration or a decreased magnesium outflow.

Acknowledgment

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References


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