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THE ROLE OF THE CAT CHOROID PLEXUS IN REGULATING CEREBROSPINAL FLUID MAGNESIUM

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SUMMARY

1. The regulation of c.s.f. Mg concentration was studied using the cat choroid plexus isolated in a chamber *in situ*.

2. An increase in plasma Mg concentration was accompanied by the usual reciprocal decrease in plasma Ca concentration. Chamber fluid Ca concentration was unaffected.

3. Hypermagnesemia (plasma Mg concentration greater than 6 m-equiv/l.) caused relatively small increases in c.s.f. Mg concentration (Δ plasma [Mg]/Δ c.s.f. [Mg] = 4).

4. Various chamber fluid Mg concentrations (0, 2·4 or 4·8 m-equiv/l.) were rapidly (within 30–60 min) returned to near the control value of 1·83 m-equiv/l.

5. When plasma and chamber fluid Mg concentrations were altered simultaneously, the final chamber fluid Mg concentration was returned towards normal with or against a concentration gradient.

6. The data indicate that the choroid plexus is involved in maintaining the constancy of the c.s.f. Mg concentration by sensing changes in the normal c.s.f. Mg concentration and altering approximately its rate of active secretion of Mg.

INTRODUCTION

It has long been known that the concentration of magnesium in the c.s.f. of mammals is maintained remarkably constant even when the concentration of magnesium in the plasma is varied (Cohen, 1927; McCance & Watchorn, 1931; Kemény, Boldizsár & Pethes, 1961; Oppelt, MacIntyre & Rall, 1963; Bradbury, Kleeman, Bagdoyan & Berberian, 1968; Olsen & Sørensen, 1971). This is of particular interest in view of the relation between the excitability of tissues and the attendant magnesium concentrations. However, the exchange of ^{28}Mg between c.s.f. and blood is rapid (Oppelt *et al.* 1963). In studies of c.s.f. as well as newly formed choroid plexus fluid it has been shown that the concentration of magnesium in these fluids is higher than that in plasma (Cohen, 1927; Ames, Sakanoue & Endo, 1964; Woodward & Reed, 1969; Miner & Reed, 1972). The mechanisms responsible for the constancy of the c.s.f. magnesium concentration and the maintenance of the magnesium gradient between c.s.f. and the plasma have not been defined. For example, it is not certain whether the choroid plexus is directly involved in the regulation of the c.s.f. magnesium concentration. Since the magnesium concentration in extracellular fluid of brain is known to affect brain excitability and it is generally believed that

c.s.f. and brain extracellular fluid are in near equilibrium, regulation of the c.s.f. magnesium concentration may be important in maintaining the appropriate concentration of magnesium in the C.N.S.

This study was undertaken to evaluate the role of the choroid plexus in regulating the magnesium concentration in the c.s.f.

METHODS

Experimental. Adult cats of either sex, body weight 2·5–3·5 kg, were anaesthetized with sodium pentobarbitone, 35 mg/kg, intraperitoneally. A tracheotomy was performed and the animal was maintained on artificial respiration throughout the experiment. The femoral artery was cannulated for monitoring blood pressure and for collecting blood samples. The femoral vein was cannulated to permit intravenous administration of supplemental anaesthetic and infusion of 105 mM-$MgCl_2$ when hypermagnesemia was desired. Body temperature was maintained near normal with an electric heating pad. The choroid plexus was isolated in a chamber *in situ* as described in detail by Miner & Reed (1972). In brief, the method requires an extensive unilateral craniotomy, removal of the cerebral cortex overlying the left lateral ventricle and exposure of the choroid plexus within the lateral ventricle. An appropriate segment of the plexus is then separated from its underlying connective tissue attachments and isolated within a chamber with the blood and nerve supply intact.

Collection of chamber fluid. After completion of the isolation procedure all the fluid in the chamber was removed. Fluid secreted by the choroid plexus was allowed to collect in the chamber under a layer of liquid petrolatum that was added to eliminate fluid evaporation. In some experiments 50 μl. artificial c.s.f. (described below) was added to the chamber at the start of the collection period. At the end of the collection period all the fluid beneath the liquid petrolatum was removed from the chamber and the collection procedure repeated as long as the preparation was stable, i.e. adequate blood pressure, plexus blood flow and fluid secretion rate. Collection periods were either 15, 30 or 60 min. An arterial blood sample (0·5 ml.) was obtained at the midpoint of each collection period.

Artificial cerebrospinal fluids. The artificial c.s.f. described by Merlis (1940) was used as the normal Mg c.s.f. (2·4 m-equiv/l.); the low Mg and high Mg fluids were prepared by interchanging NaCl and $MgCl_2$ in the Merlis c.s.f. so that the final magnesium concentrations were zero and 4·8 m-equiv/l., respectively.

Analysis. The protein-free plasma supernatant was prepared by adding 4 ml. 0·5 N-HNO_3 to 0·2 ml. plasma which was then centrifuged at 3000 rev/min. 10 μl. chamber fluid was diluted (1 : 1000) or 1 ml. plasma supernatant (1 : 10) with La-Sr solution ($1·8 \times 10^{-2}$ M-La_2O_3; $1·1 \times 10^{-4}$ M Sr $Cl_2.6H_2O$) for determination of Mg and Ca by atomic absorption spectrophotometry. Protein concentrations were determined by the method of Pesce & Strande (1973) on 20 μl. samples of chamber fluid and a comparable volume of 1 : 20 dilution of whole plasma.

Calculations. Concentrations of Mg and protein in the newly formed c.s.f. were calculated by subtracting the amounts of the substance initially added to the chamber in the artificial c.s.f. from the total amount in the chamber fluid and dividing by the volume of new c.s.f. secreted during the collection period. Thus it is assumed that any change in chamber fluid concentration was due to the addition of the measured volume of new fluid of the calculated composition. This may not be absolutely correct but it is not important for the purpose of this presentation whether the change in the quantity of the substance in the chambers is expressed as a flux or as a change in concentration in the new fluid. The formula used is:

$$\text{new c.s.f. } [X] = \frac{[X]_{cf} V_{cf} - [X]_a V_a}{V_{cf} - V_a},$$

where $[X]$ is the concentration of the substance (Mg or protein) in the fluid, V is the fluid volume and the subscripts 'cf' and 'a' refer to chamber fluid and added artificial c.s.f., respectively.

Free Mg concentration. Since atomic absorption spectrophotometry which was used to measure total Mg in all samples does not differentiate between bound and free Mg, it was necessary to determine the fraction of plasma magnesium that was free and this was measured by pressure dialysis. This value was used to calculate the plasma free Mg concentration.

RESULTS

As determined by graphic analysis of the data (Fig. 1) there was a significant ($P < 0{\cdot}05$) decrease in plasma Ca concentration as the plasma Mg concentration increased. The effects of magnesium infusion on plasma and c.s.f. Ca concentrations were determined by linear regression analysis (Snedecor & Cochran, 1967). The slope of the line relating plasma Ca to plasma Mg is $-0{\cdot}08 \pm 0{\cdot}04$ (S.D.) and $r = -0{\cdot}04$. For chamber c.s.f. Ca versus plasma Mg the slope is $0{\cdot}1 \pm 0{\cdot}04$ ($r = 0{\cdot}03$, $P > 0{\cdot}05$; see Fig. 1). Thus, there was no effect of altered plasma Mg concentration on c.s.f. Ca concentrations (and also the rate of fluid formation was not affected).

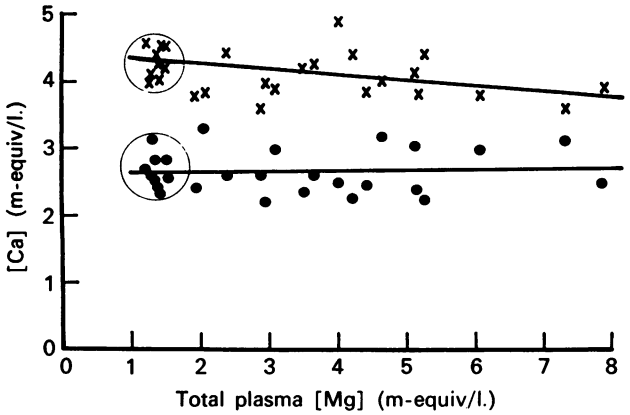

Fig. 1. The relation between Mg and Ca in plasma (× — ×) and chamber fluid (●—●). Each point represents the value for Ca or Mg in one 30 min collection period. The lines were fitted to the data by linear regression analysis. The encircled values represent controls.

The average rate of fluid accumulation in the chamber was 1·58 μl./min. The wet weight of the choroid plexus within the chamber was approximately 3–4 mg. On the basis of the average rate of fluid accumulation and the wet weight of plexus in the chamber, the rate of c.s.f. production by the plexus was about 0·4–0·5 μl./min.mg. Mean protein concentration of the new c.s.f. was 0·68 g/100 ml. (range 0·07–1·59 g/100 ml.). These values for fluid production and protein concentration are similar to those previously reported (Miner & Reed, 1972; Husted & Reed, 1976).

The relationship of the Mg concentration of chamber fluid to that of an ultrafiltrate of plasma (plasma free Mg) is shown in Fig. 2. Each point on the graph represents the value of one 30 min collection period; usually two collections were made for each animal. In evaluating the relationship between plasma and c.s.f. Mg concentrations it is important to use the plasma-free Mg concentration since the bound form appears not to cross the choroid plexus. The fraction of Mg bound when measured over a wide range of plasma Mg concentrations was not related to the plasma Mg concentration over the range observed in this study. The mean value for protein binding of Mg in plasma was 25 %. Therefore, the factor of 75 % was used to calculate the free Mg in all plasma samples. The line in Fig. 2 was calculated

by linear regression. The slope of the line is $0{\cdot}25 \pm 0{\cdot}04$, $r = 0{\cdot}78$. The Mg concentration of the chamber c.s.f. increased about 0·25 m-equiv/l. for each 1 m-equiv/l. change in the plasma concentration of Mg in response to the infusion of 1 % $MgCl_2$. The encircled values represent measurements on control animals and it can be seen that the chamber fluid c.s.f. Mg concentration is significantly higher than the free Mg concentration of plasma ($1{\cdot}83 \pm 0{\cdot}03$ *vs.* $1{\cdot}08 \pm 0{\cdot}04$ m-equiv/l.)

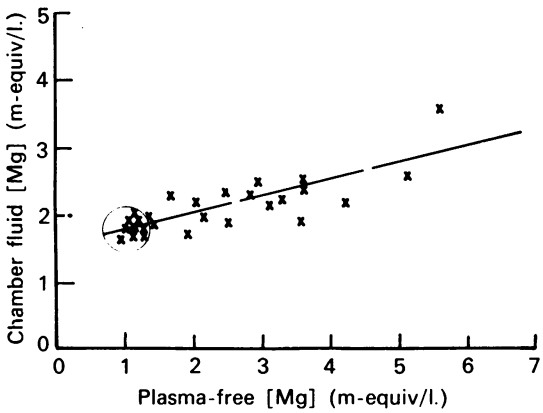

Fig. 2. The relationship of free Mg concentration in chamber fluid and in plasma. Each point represents the results of a 30 min collection; usually at least two collections were made in each animal. The encircled values represent controls. The line was determined by regression analysis. The slope is significantly less than one. The data were obtained from nine cats.

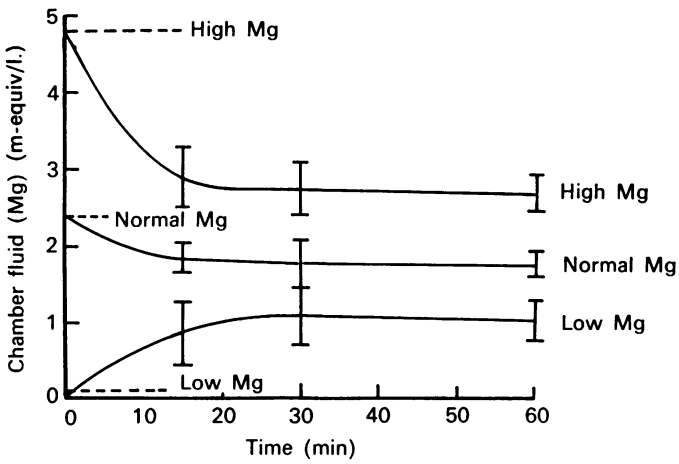

Fig. 3. The concentration of Mg in the chamber fluid as a function of time after altering the chamber fluid c.s.f. Mg concentration. The dashed lines indicate the concentration of Mg in the chamber fluid that was added to the chamber initially. (Low Mg = 0 m-equiv/l.; normal Mg = 2·4 m-equiv/l.; high Mg = 4·8 m-equiv./l.). Vertical bar = mean $\pm$ 1 S.E.

The time course of the response of the choroid plexus to various concentrations of Mg was determined by adding 50 μl. of artificial c.s.f. solution that contained one of three different concentrations of Mg (low = 0, normal = 2·4, high = 4·8 m-equiv/l.)

into the chamber with no alteration in plasma Mg, after which c.s.f. was collected for various times. The results are shown in Fig. 3. Within 15 min the major correction of both the high and the low chamber fluid concentration of Mg has occurred and the values remain constant after 30 min.

The relationship of the chamber fluid Mg concentration to the plasma-free Mg as a function of the initial chamber fluid Mg concentration is shown in Fig. 4. The

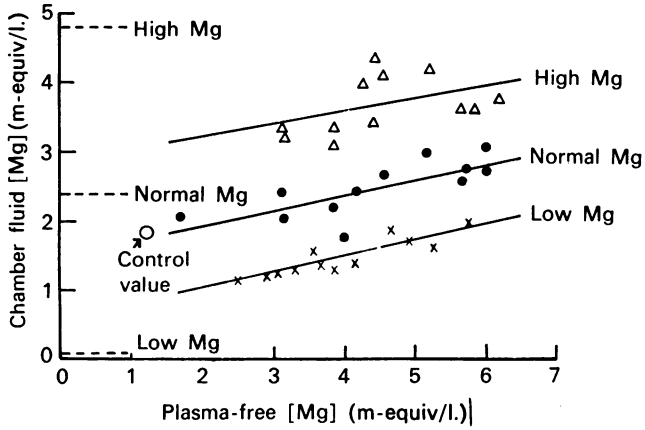

Fig. 4. Regression of the concentration of Mg in the chamber fluid 30 min after addition of the artificial c.s.f. with various Mg concentrations upon plasma Mg concentration. The three parallel lines were determined by linear regression analysis. The chamber fluid Mg concentrations are the same as in Fig. 3.

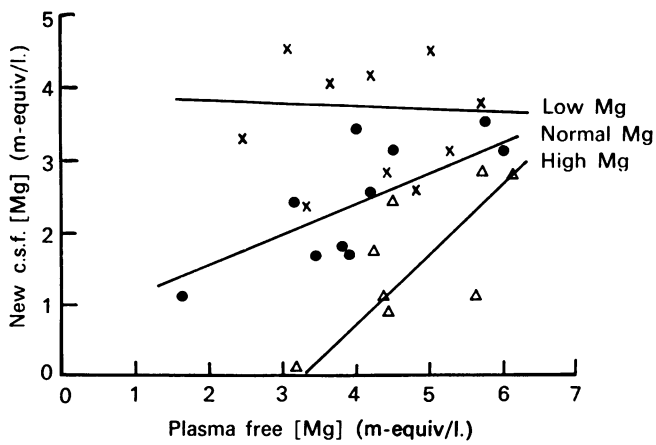

Fig. 5. Regression of the calculated newly formed c.s.f. Mg concentrations upon plasma free Mg concentration after addition of the various artificial c.s.f.

dashed lines, labelled high Mg, normal Mg and low Mg, show the concentration of Mg in the various artificial c.s.f. solutions that were added to the chamber initially. Each point on the graph represents the Mg concentration in the fluid in the chamber at the end of a 30 min collection period and usually two collection periods with each added artificial c.s.f. were obtained from each animal. The continuous lines were

calculated by analysis of covariance (Snedecor & Cochran, 1967). The combined slope of the parallel lines is $0{\cdot}21 \pm 0{\cdot}05$. The two lines representing the chamber fluid Mg concentration 30 min after introducing the high or low Mg fluid into the chamber are significantly displaced from the normal Mg curve ($P < 0{\cdot}01$). It can also be seen that the effect of increasing the plasma free Mg concentration on the chamber fluid Mg is apparent regardless of the initial chamber fluid Mg concentration.

The calculated Mg concentration in the new c.s.f. formed during the collection period is plotted versus plasma free Mg concentration in Fig. 5. The plotted points are derived from the values shown in Fig. 4 by use of the formula given in Methods. The calculated Mg concentration in the newly formed fluid varied inversely with the Mg concentration of the fluid added to the chamber. It is also apparent that the lines are not parallel as they are in Fig. 4.

DISCUSSION

Perhaps the most widely used experimental methods to study the regulation of c.s.f. constituents have been ventriculocisternal (v.c.) perfusion, *in vitro* choroid plexus incubation and c.s.f. sampling under various conditions. With the v.c. perfusion technique and with c.s.f. sampling the effects of varying plasma and c.s.f. Mg can be studied but the site at which regulation occurs cannot be determined. Bradbury (1965) found that when varying concentrations of Mg were added to the v.c. perfusion fluid the concentration in the fluid collected at the cisterna magna had been altered toward the normal value.

The technique used by Ames *et al.* (1964), which involved collection of the fluid formed *in situ* by the exposed choroid plexus of the lateral ventricle, demonstrated that the concentration of Mg in the c.s.f. is higher than that in plasma. However, it has been suggested that the fluid collected by this method might be of extra-choroidal origin (Welch, 1963) and therefore, the role of the choroid plexus in c.s.f. Mg regulation remains to be elucidated.

The *in situ* isolated choroid plexus technique developed by Miner & Reed (1972) permits the study of the composition of the fluid formed by the choroid plexus with a normal blood and nerve supply without the possibility of alteration by non-choroidal tissues. The use of this technique in the present study demonstrates that the homeostasis of Mg in c.s.f. is regulated, at least in part, by the choroid plexus.

In view of the reciprocal relation between Mg and Ca frequently observed in mammalian body fluids, the effect of varying plasma Mg concentration on Ca concentration in plasma and in c.s.f. were determined. From the data (Fig. 1) it can be seen that the concentration of Ca in c.s.f. is maintained constant even when plasma Ca is significantly decreased concomitant with the increase in the plasma Mg levels. Morgulis & Perley, as early as 1930, studied the relation of c.s.f. Ca to serum Ca in man and in dog and found that when the serum Ca was greatly increased by means of a continuous I.V. infusion of a modified Ringer solution containing a large amount of $CaCl_2$ the c.s.f. Ca remained constant. This suggests that perhaps other mechanisms besides passive diffusion are operating in Ca regulation. The ability to regulate Ca in a normal fashion in the present study suggests that the experimental conditions have not altered significantly the ability of the choroid plexus in the chamber to function normally.

From the data shown in Fig. 2 it is apparent that the chamber fluid Mg concentration under control conditions is higher than that of free Mg in plasma. Similar observations have been made for c.s.f.–plasma relationships. This suggests that the choroid plexus actively transports Mg into the c.s.f. to produce the gradient. Held, Fencl & Pappenheimer (1964) observed a potential of +6 mV between the c.s.f. and the blood in the anaesthetized goat and dog. The electrical potential across the choroid plexus in the present experiments was not measured. However, Husted & Reed (1976) measured the potential in the choroid plexus preparation *in situ* and found transchoroidal potentials of +2 to 5 mV. The existence of such potentials in the present experiments would provide additional support for active transport since Mg would be moving against both an electrical and a chemical gradient.

It can also be seen from Fig. 2 that when the plasma-free Mg concentration reaches about 2 m-equiv/l. it is in chemical equilibrium with the chamber fluid Mg. Further increases in the plasma Mg concentration actually reverse the normal (c.s.f. to plasma) Mg gradient and yet the chamber fluid Mg concentration increases only at about a quarter of the rate of the plasma free Mg. This would suggest that either the choroid plexus is only slightly permeable to Mg and the normal inward transport of Mg is markedly reduced or the membranes are freely permeable to Mg and the direction of Mg transport is reversed. The former seems more likely.

As shown in Fig. 3, when either high or low concentrations of Mg are added to the chamber the concentration of Mg in the chamber fluid rapidly returns toward the normal value. This suggests that the choroid plexus cannot only detect abnormal Mg concentration in the c.s.f. but also can respond promptly and appropriately to restore the concentration toward normal. Of additional interest is the observation that the Mg concentration does not return completely to the control value immediately but appears to approach it asymptotically. This is probably due to inadequate stirring of the chamber fluid. The choroid plexus is a ciliated epithelium whose cilia beat at a rapid rate. It has been postulated that the actions of the cilia are responsible in part for circulating or mixing the c.s.f. (Davson, 1967). In the case of the cat brain the depth of the layer of c.s.f. adjacent to the choroid plexus is very thin compared to that in the chamber. Thus the composition of the layer of fluid immediately adjacent to the choroid plexus in the chamber may be significantly different from the bulk of the chamber fluid if the action of the cilia or other factors are inadequate to provide good mixing of the chamber contents. The fluid layer in contact with the choroid plexus may in fact closely resemble normal c.s.f. which would not provide a stimulus for further altered Mg transport. The time for diffusion over the distances encountered in the chamber in an unmixed solution would be long and may thus explain the observation.

The data in Fig. 4 resulted when both the plasma and the chamber fluid Mg concentrations were altered. It is significant that a comparison of the values obtained when normal Mg artificial c.s.f. was added to the chamber are not different from those presented in Fig. 2 when no fluid was added to the chamber initially. This clearly demonstrates that the mere addition of fluid to the chamber did not affect the function of the choroid plexus tissue in regulating chamber fluid Mg. Also of interest is the fact that the curves obtained with the different initial Mg concentrations are parallel with a slight positive slope. This observation could be explained

if, in the case of the high Mg curve, the blood to chamber fluid Mg pump was turned off by the high Mg concentration in the chamber fluid with no detectable effect on the rate of fluid secretion and there was a concurrent slow passive leak of Mg into the chamber fluid proportional to the plasma free Mg concentration. Similarly, the low Mg curve would be predicted if the same passive leak existed but the Mg pump was stimulated by the low Mg concentration in the chamber fluid.

From the calculated concentrations of Mg in newly formed fluid shown in Fig. 5, it is apparent that the Mg concentration in the newly formed fluid is the highest when low Mg artificial c.s.f. is added to the chamber; least when the high Mg is added and intermediate when normal Mg fluid is present. The mechanism suggested above to explain the observations presented in Fig. 4 are compatible with the data shown in Fig. 5. Thus, when low Mg fluid was placed in the chamber the Mg pump is maximally stimulated and this would represent the major source of Mg entering the chamber. Since diffusion would make only a small contribution to the total Mg entering the chamber, a practically horizontal line would be expected. Conversely, when high Mg fluid was added to the chamber and the pump is 'turned off' the change in concentration of the chamber fluid would depend primarily on the diffusion component. Therefore, Mg would not enter the chamber until the plasma-free Mg concentration exceeded that in the chamber which apparently occurred at between 3 and 4 m-equiv/l. in the plasma as indicated by the high Mg curve. As the diffusion gradient increased, the Mg concentration of the new fluid would also increase. The normal Mg line would be similar to those previously presented (Figs. 2 and 4).

In summary, this study demonstrates clearly that the choroid plexus can detect alterations in the Mg concentration of the c.s.f. in its immediate environment. Furthermore, it can alter its function appropriately to return the Mg concentration of the fluid on the c.s.f side toward normal. The data presented are compatible with the hypothesis that this is accomplished by the active transport of Mg by a system whose rate of transport from blood to c.s.f. can be varied appropriately and that the transporting tissue has a real but limited permeability to Mg. This system may be important in the regulation of c.s.f. and C.N.S. Mg concentrations.

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