Responses to the Lowering of Magnesium and Calcium Concentrations in the Cerebrospinal Fluid of Unanaesthetized Sheep

T. F. Allsop and J. V. Pauli

Wallaceville Animal Research Centre, Research Division, Ministry of Agriculture and Fisheries, Private Bag, Upper Hutt, New Zealand.

Abstract

A technique for ventriculolumbar perfusion of the cerebrospinal fluid space has been used to study the neuromuscular effects of low concentrations of magnesium and calcium in the cerebrospinal fluid of conscious sheep. Perfusion with synthetic cerebrospinal fluid solutions containing less than 0.6 mg magnesium/100 ml produced episodes of tetany which were abolished by perfusion with a solution of normal magnesium concentration. This suggests that the low cerebrospinal fluid magnesium concentrations reported in cases of hypomagneseamic tetany may result in changes within the central nervous system that could produce the nervous signs. Perfusates with a calcium concentration below 2.0 mg/100 ml caused hyperpnoea and continuous muscle tremors. Magnesium (0.6 mg/100 ml) and calcium (2.0 mg/100 ml) perfused simultaneously acted synergistically to produce signs characteristic of low levels of each of the ions.

Introduction

The magnesium concentration of cerebrospinal fluid (CSF) is normally closely controlled, even in the presence of wide variations in plasma magnesium concentrations (Oppelt *et al.* 1963*a*; Meyer and Scholz 1972). Magnesium is thought to be accumulated from the blood to the CSF by an active transport mechanism in the choroid plexus and removed by diffusion and bulk filtration through the arachnoid villi, with a half-life, in dogs, of about 70 min (Oppelt *et al.* 1963*a*).

The signs of tetany in severely hypomagnesaemic sheep and dairy cows are associated with low CSF magnesium concentrations (Meyer and Scholz 1972; Pauli and Allsop 1974). Verstraeten (1950) and Leusen (1950) perfused anaesthetized dogs ventriculocisternally for 15 min with a synthetic CSF containing no magnesium and could find no consistent effect on the respiration rate, cardiac output or blood pressure. To indicate whether the nervous signs of hypomagnesaemic tetany may be due to low CSF magnesium concentrations, the effect of low CSF magnesium levels was studied in sheep with normal plasma magnesium concentrations.

The effects of lowering CSF calcium levels alone or in conjunction with lowered CSF magnesium were also studied. In anaesthetized dogs, Verstraeten (1950) showed that lowering the CSF calcium caused profound hyperpnoea but normally CSF calcium is closely regulated despite wide variations in plasma calcium concentration (Oppelt *et al.* 1963b).

Materials and Methods

Animals and Surgical Preparation

Eight, four-tooth or older, non-pregnant Romney ewes, fed a pelletted lucerne diet, were used-Preliminary surgical preparation consisted of implanting a permanent guide for cannulation of the lateral ventricle of the brain by a modification of the method of Pappenheimer *et al.* (1962). Surgery was performed under halothane anaesthesia after induction with intravenous sodium thiopentone. A longitudinal incision was made in the skin covering the cranium so that a $2 \cdot 2$ mm diameter hole could be drilled perpendicularly through the exposed bone, $0 \cdot 5$ cm from the midline and $3-3 \cdot 5$ cm caudal to a line joining the caudal limits of the bony orbits. A truncated 13-gauge needle with a 4-mm shaft was fitted tightly into the hole and a 5-cm, 16-gauge needle inserted through the guide until ventricular CSF welled up through it. The depth was noted. The needle was then withdrawn and the guide plugged with a rubber septum. The junction between the guide hub and the bone was then sealed with a few drops of tissue glue (COAPT Gewebekleber, Ethicon GmbH, Hamburg, Germany) and the guide supported by a mound of dental acrylic cement (S. S. White Dental Mfg Co. (G.B.) Ltd, England). The skin incision was then sutured close around the guide. Sheep with this preparation have been used intermittently for a period of 3 months.

Synthetic Perfusion Fluid

A synthetic, control CSF was prepared in distilled, pyrogen-free water and made up to 1 litre. The composition is shown in the following tabulation:

Ions	mg/100 ml	Salt	g/l
Na+	342	NaCl	7.200
K+	12.1	KC1	0.230
Ca ²⁺	5.4	CaCl ₂ .2H ₂ O	0.2119
Mg ²⁺	2.4	MgCl ₂ .6H ₂ O	0.2011
Cl-	466	2	• 2011
HCO3 -	122	NaHCO ₃	1.680
Inorganic P	1.55	NaH ₂ PO ₄ .2H ₂ O	0.078
Glucose	70	Glucose	0.700

The concentrations of magnesium and calcium were changed for the experimental perfusions to provide solutions containing one of the following concentrations (mg/100 ml): $Mg^{2+} 0.0 \text{ or } 0.6$; $Ca^{2+} 0.0 \text{ or } 1.0 \text{ or } 2.0$; $Mg^{2+} 0.6$ and $Ca^{2+} 1.0$; $Mg^{2+} 0.6$ and $Ca^{2+} 2.0$.

The solutions were sterilized using a bacteriological filter (0.2 μ m pore size) (Sartorius Membranfilter GmbH, Göttingen, Germany).

Perfusion Technique

The sheep was restrained in a sling and the lateral ventricle was cannulated. A lumbar puncture was performed with a 5-cm, 16-gauge, short-bevel needle.

The synthetic CSF was maintained at 39°C and bubbled with a mixture of 95% O_2 and 5% CO_2 (resultant pH 7·4). The solution was pumped into the lateral ventricle using a peristaltic pump and sterile tubing assembly with a manometer side-arm similar to that described by Pappenheimer *et al.* (1962). The flow was adjusted to the required rate and the input pressure was maintained at less than 20 cm water above the outflow level.

The outflow tube was attached to the lumbar needle and the outlet maintained level with the sheep's back. Ten-minute fractions of the outflow solution were collected. In experiments when the recovery of perfused fluid was less than 80% the results were discarded.

Blood and lumbar CSF samples were taken from each sheep prior to perfusion. Hourly blood samples were taken by way of an indwelling, jugular cannula during three perfusions. Lithium heparin (143 USP units/10 ml blood) was added to all blood samples as an anticoagulant.

Analysis

Plasma, CSF and perfusion fluid were analysed for magnesium and calcium by atomic absorption spectroscopy by the method of Willis (1960) using 0.75% (w/v) EDTA.

Results

A flow rate of 1.5 ml/min was used for the initial perfusions (Table 1). In these initial experiments the sheep were left to recover from tetany without the use of complete CSF solution and the time taken was noted. Results obtained with a flow rate of 1.0 ml/min are also given in Table 1. In all but two of these experiments the complete solution was perfused following production of severe signs. The signs were completely abolished by the time the outflow magnesium or calcium or both magnesium and calcium concentrations had returned to about normal. Fig. 1 presents results obtained from one sheep (No. 3) under various perfusion conditions.

Table 1. Times for the production and resolution of signs produced by the perfusion of synthetic CSF solutions containing various concentrations of magnesium and calcium and the outflow concentrations of magnesium and calcium at these times

Concentrations are expressed as milligrams per 100 ml. Time (min) is the time to the appearance of signs (mild or severe) or the time to recovery (no signs)

Concn in sperfusion solution	Sheep	Mild signs		Severe signs		Time	No signs Outflow concn		Total perfusion		
		(min)	Mg ² +	Ca ² +	(min)	Mg ² +	Ca ² +	(min)	Mg ² +	Ca ² +	time (min)
Complete	1 ^A								2·2 ^в	5·4 ^B	600
	1 ^A								2·3 ^в	5·4 ^в	600
	2								2·3 ^в	5·0 ^B	480
	3								2·4 ^в	5·0 ^B	600
	4								2·4 ^в	5·2 ^в	600
Magnesium 0·0	1 ^A	270	0.32	5.2	570	0.25	5 · 1	730 ^c			670
	2 ^A	170	0.39	5.3	260	0.36	5.3	340 ^c			270
	3	150	0.40	$5 \cdot 1$	280	0.38	5.1	350	2.2	5 · 1	430
	4	350	0.48	5.0	560	0.40	5.0	640	$2 \cdot 1$	5 · 1	670
Magnesium 0·6	2	225	0.90	5.4				735	2.8	5.0	760
	3		0·88 ^в	5·4 ^в							720
	5		0·89 [₿]	5∙4 [₿]							600
	6	_	0∙88 [₿]	5 · 5 ^B							370
Calcium 0.0	2	10	2.6	4.6	60	2.7	0.89	140	2.7	4.6	160
	7	20	2.4	$5 \cdot 2$	50	$2 \cdot 2$	$1 \cdot 8$	200 ^c			60
Calcium 1.0	3	30	2.5	3.6	100	2.5	2.0	160	$2 \cdot 6$	4.4	170
	8	40	2.5	2.1	150	2.5	$1 \cdot 6$	300	$2 \cdot 5$	4.8	350
Calcium 2.0	2	150	$2 \cdot 8$	2.7				310	2.7	4.5	320
	3	430	2.5	$2 \cdot 5$				600	$2 \cdot 6$	4.9	600
	5		2·5 ^в	2·5 ^в							500
Magnesium 0.6 and calcium 1.0	3	10	2.4	5.0	30	1.4	2.9	130 ^c			60
Magnesium 0.6	3	40	1.6	4·0	160	0.94	2.7	300	2.2	4.9	320
and calcium $2 \cdot 0$) 5	50	1.1	2.8	210	0.93	2.5	300	2.1	4.6	330
	6	40	1.9	4·3	130	0.87	2.1	280	2.2	4.6	290

^A Perfused at 1.5 ml/min, remainder all at 1.0 ml/min.

^B Minimum outflow concentration attained in animals showing no signs.

^c Complete solution not perfused to resolve signs.

Blood samples taken from all the sheep prior to perfusion had a mean plasma magnesium concentration (\pm s.E.) of $2 \cdot 32 \pm 0 \cdot 34 \text{ mg}/100 \text{ ml}$ and a mean calcium concentration of $9 \cdot 79 \pm 0.65 \text{ mg}/100 \text{ ml}$. Samples of CSF taken at the same time had a mean magnesium concentration of $2 \cdot 38 \pm 0.14 \text{ mg}/100 \text{ ml}$ and a mean calcium concentration of $5 \cdot 16 \pm 0.42 \text{ mg}/100 \text{ ml}$.

Control Perfusions

Sheep perfused at both flow rates for up to 600 min with complete, synthetic CSF solution showed no signs. The outflow concentrations remained between $2 \cdot 2$ and $2 \cdot 4 \text{ mg}/100 \text{ ml}$ for magnesium and between $5 \cdot 0$ and $5 \cdot 4 \text{ mg}/100 \text{ ml}$ for calcium.

Low-magnesium Perfusions

(a) 0.0 mg magnesium/100 ml

The first mild signs to appear were episodes of up to 10 s duration involving one or more of the following: fine muscle tremors of the head and neck, tetanic extension of the neck and forelegs, and paddling, mainly with the forelegs. During the intervals of 10 min or more between these episodes the sheep appeared normal. The outflow magnesium concentration had fallen to a mean value of 0.40 mg/100 ml by the time mild signs appeared.



Fig. 1. Outflow magnesium (----) and calcium (---) concentrations for sheep No. 3 perfused with (a) 0.6 mg magnesium/100 ml, (b) 0.0 mg magnesium/100 ml, (c) 1.0 mg calcium/100 ml (I) or 2.0 mg calcium/100 ml (II), and (d) 0.6 mg magnesium/100 ml and 2.0 mg calcium/100 ml simultaneously. Arrows mark the time of connection of the complete perfusion solutions. Light hatching on the bars represents mild signs and dense hatching represents severe signs.

The signs were regarded as severe when the episodes involved tetanic extension of the neck and all legs for periods of greater than 10 s and were followed by violent paddling of all the legs. The intervals between episodes were 10 min or less and in some cases the episodes became almost continuous, producing tetany lasting for up to 2 min. At this stage the outflow magnesium concentration had fallen to a mean value of 0.35 mg/100 ml.

(b) 0.6 mg magnesium/100 ml

In one of four sheep, mild signs were produced involving extension of the neck and legs with some paddling in episodes of 5-10 s duration. The signs continued throughout the perfusion period but were not severe. The mean outflow magnesium concentration for all the sheep fell to 0.89 mg/100 ml.

Low-calcium Perfusions

(a) $0 \cdot 0$ mg calcium/100 ml

The first signs to appear were fine, muscle tremors of the whole body, an appearance of extreme alertness, and an increase in the respiration rate. The outflow calcium concentration had fallen to a mean of 4.9 mg/100 ml at this time but the calcium level of the fluid surrounding the brain was undoubtedly much lower.

The sheep rapidly became hyperactive and the signs were considered severe when rapid and heavy breathing through the mouth began. The signs were not in discrete episodes but were continuous, and increased in severity with time. The mean outflow calcium concentration was $1 \cdot 3 \text{ mg}/100 \text{ ml}$ at the onset of severe signs.

(b) $1 \cdot 0$ mg calcium/100 ml

The signs were similar in nature and severity to those described for 0.0 mg calcium/100 ml, but took longer to appear and to progress to the severe stage The mean outflow calcium concentration had fallen to 2.9 mg/100 ml by the time mild signs appeared and to 1.8 mg/100 ml with severe signs.

(c) $2 \cdot 0 \text{ mg calcium}/100 \text{ ml}$

The first signs to appear in two out of three sheep were rapid breathing through the nose only and some increased alertness and activity. The mean outflow calcium concentration for all the sheep was $2 \cdot 6 \text{ mg}/100 \text{ ml}$. The signs did not increase in severity with time. One sheep showed no signs but did not have a significantly different outflow calcium concentration from those that did show signs.

Low-magnesium and Low-calcium Perfusions

(a) 0.6 mg magnesium/100 ml and 1.0 mg calcium/100 ml

The signs produced were similar to those resulting from perfusion with 0.0 mg calcium/100 ml solution. Mild signs appeared when the magnesium and calcium outflow concentrations were 2.4 and 5.0 mg/100 ml respectively and rapidly developed into severe signs by the time the respective concentrations had reached 1.4 and 2.9 mg/100 ml.

(b) 0.6 mg magnesium/100 ml and 2.0 mg calcium/100 ml

The early signs were similar to those produced by the 1.0 mg calcium/100 ml solution. At this stage the outflow magnesium and calcium concentrations had fallen to mean values of 1.5 and 3.7 mg/100 ml respectively. As the signs became more severe, tetanic episodes characteristic of low CSF magnesium concentrations occurred in conjunction with the calcium deficiency signs. The mean outflow concentration of magnesium was 0.91 mg/100 ml and that of calcium was 2.8 mg/100 ml at this stage.

Changes in Plasma Magnesium and Calcium Concentrations

Hourly blood samples were taken from one sheep (No. 4) during perfusions with complete solution and with 0.0 mg magnesium/100 ml solution. During the control perfusion the mean plasma magnesium concentration (\pm s.e.) was $2.01 \pm 0.04 \text{ mg}/100$ ml and the mean calcium concentration was $8.76 \pm 0.15 \text{ mg}/100$ ml. The mean

plasma magnesium and calcium concentrations during the four stages of the zero magnesium perfusion were respectively: prior to the onset of signs 1.92 ± 0.06 and 9.61 ± 0.10 mg/100 ml; during mild signs 1.85 ± 0.04 and 9.55 ± 0.06 mg/100 ml; during severe signs 1.90 and 9.72 mg/100 ml; after recovery following perfusion with complete CSF solution 1.95 and 9.97 mg/100 ml.

Blood samples were also taken during perfusion of sheep No. 7 with 0.0 mg calcium/100 ml solution. The magnesium concentration of the plasma remained at the initial level of 1.68 mg/100 ml during severe signs and the calcium concentration changed from an initial value of 9.36 mg/100 ml to 9.48 mg/100 ml during severe signs.

Discussion

Removal of magnesium from the CSF of conscious sheep produced characteristic neuromuscular signs that were not unlike those of hypomagnesaemic tetany. The effect was apparently not mediated through any large changes in blood magnesium concentration. All of the sheep were sensitive to depletion of CSF magnesium but the degree of susceptibility, as measured by the time of onset of signs or by the concentration of magnesium in the outflow solution, varied markedly between animals. The time taken to produce signs is probably a function of the time required to lower the magnesium concentration of the interstitial fluid of the central nervous system which is separated from the CSF by a permeable barrier (Rall *et al.* 1962).

The initial sharp fall in outflow magnesium concentration (Fig. 1b) was due to the rapid removal of native CSF by the perfusate. Thereafter the rate of fall in outflow magnesium concentration was much less as the magnesium accrued from further CSF synthesis and from depletion of extracellular fluid magnesium. It is likely that once the latter reaches a critical concentration there is a change in the neuronal membranes resulting in increased excitability and consequent tetany (Leusen 1972).

Under the perfusion conditions used here a level of 0.6 mg magnesium/100 ml in the perfusate would seem to be a marginal concentration for the production of signs within 10 h. This concentration is lower than that observed in sheep with hypomagnesaemic tetany (Meyer and Scholz 1972), but in that case the duration of exposure to low CSF magnesium concentrations was probably greater and the magnesium concentration of the plasma was invariably low. It appears, however, that the low CSF magnesium concentrations reported in cases of hypomagnesaemic tetany may be responsible for a change in the magnesium concentration of some compartment of the central nervous system, possibly in the extracellular fluid, which may cause the nervous signs.

Removal of calcium from the CSF also caused characteristic signs, which were distinct from those induced by lack of CSF magnesium. A calcium concentration of $2 \cdot 0 \text{ mg}/100 \text{ ml}$ in the perfusion fluid appeared to be about the maximum level for production of signs within 10 h. Again it is likely that the effects are due to removal of calcium from the extracellular fluid of the central nervous system.

The simultaneous removal of magnesium and calcium from the CSF exacerbated the effects of their removal individually. A perfusate containing 0.6 mgmagnesium/100 ml and 2.0 mg calcium/100 ml produced severe signs characteristic of the lack of both ions, whereas when perfused separately at these respective concentrations the signs were no more than mild. Magnesium and calcium have been shown to act synergistically to stabilize the neural membranes of myelinated nerve fibres (Frankenhaeuser and Hodgkin 1957; Frankenhaeuser and Meves 1958) with calcium having the greater stabilizing effect. This is in contrast to the antagonistic action of these two ions at peripheral synapses (Elmqvist and Feldman 1965).

Acknowledgments

We thank Mr P. N. Trevethick for technical assistance.

References

- Elmqvist, D., and Feldman, D. S. (1965). Calcium dependence of spontaneous acetylcholine release at mammalian motor nerve terminals. J. Physiol. (Lond.) 181, 487–97.
- Frankenhaeuser, B., and Hodgkin, A. L. (1957). The action of calcium on the electrical properties of squid axons. J. Physiol. (Lond.) 137, 218-44.
- Frankenhaeuser, B., and Meves, H. (1958). The effect of magnesium and calcium on the frog myelinated nerve fibre. J. Physiol. (Lond.) 142, 360-5.
- Leusen, I. (1950). The influence of calcium, potassium and magnesium ions in cerebrospinal fluid on vasomotor system. J. Physiol. (Lond.) 110, 319-29.
- Leusen, I. (1972). Regulation of cerebrospinal fluid composition with reference to breathing. *Physiol. Rev.* 52, 1–56.
- Meyer, H. and Scholz H. (1972). Research into the pathogenesis of hypomagnesaemic tetany. I. Relationships between the magnesium content of blood and cerebrospinal fluid in sheep. Dtsch Tieraerztl. Wochenschr. 79, 55-61.
- Oppelt, W. W., MacIntyre, I., and Rall, D. P. (1963a). Magnesium exchange between blood and cerebrospinal fluid. Am. J. Physiol. 205, 959-62.
- Oppelt, W. W., Owens, E. S., and Rall, D. P. (1963b). Calcium exchange between blood and cerebrospinal fluid. Life Sci. 8, 599-605.
- Pappenheimer, J. R., Hersey, S. R., Jordan, E. F., and Downer, J. deC. (1962). Perfusion of the cerebral ventricular system in unanaesthetized goats. Am. J. Physiol. 203, 763-74.
- Pauli, J. V., and Allsop, T. F. (1974). Plasma and cerebrospinal fluid magnesium, calcium and potassium concentrations in dairy cows with hypomagnesaemic tetany. N.Z. Vet. J. 22, 227-31.
- Rall, D. P., Oppelt, W. W., and Patlak, C. S. (1962). Extracellular space of brain as determined by diffusion of inulin from the ventricular system. Life Sci. 2, 43-8.
- Verstraeten, J. M. (1950). Influences de la concentration en ions potassium, calcium et magnesium du liquide cephalo-rachidien sur la respiration. *Rev. Belge Pathol. Med. Exp.* 20, 1–21.
- Willis, J. B. (1960). The determination of metals in blood serum by atomic absorption spectroscopy.II. Magnesium. Spectrochim. Acta 16, 273–8.

Manuscript received 16 December 1974

