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Effects of Cerebral Intraventricular Magnesium Injections and a Low Magnesium Diet on Nonspecific Excitability Level, Audiogenic Seizure Susceptibility and Serotonin[1]

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BUCK, D R , A W MAHONEY AND D G HENDRICKS *Effects of cerebral intraventricular magnesium injections and a low magnesium diet on nonspecific excitability level, audiogenic seizure susceptibility and serotonin* PHARMAC BIOCHEM BEHAV 10(4) 487–491, 1979 —Cerebral intraventricular injections of 17 and 50 μg magnesium in artificial cerebrospinal fluid progressively depressed the Non-specific Excitability Level of permanently cannulated 150–200 g rats fed a low magnesium diet for 21 days Compared with control diet, a low Mg diet caused an increase in brain serotonin Weanling rats fed a low Mg diet for 2 weeks, then injected with 25 μg Mg via acute cannulations, displayed reduced audiogenic seizure susceptibility compared with sham-injected controls

Magnesium deficiency Magnesium pharmacology Serotonin Brain Behavior Intraventricular

IT IS well known that magnesium deficiency leads to hyperexcitability in several species including man, and conversely, that magnesium may be the therapy of choice for managing certain convulsive disorders The effects of magnesium on epileptic foci have been studied and pertinent literature has been reviewed [2]

Earlier research has provided some evidence that the mechanism whereby Mg deficiency acts to induce hyperexcitability lies in the central nervous system (CNS) rather than peripherally Belknap, *et al* [1] observed that supracortical injections containing 22 μg Mg as sulfate reduced the seizure activity of Mg deficient mice, while serum Mg levels remained low (about 0 7 mEq/l) We [4] found that audiogenic seizure susceptibility occurs in rats if cerebrospinal fluid (CSF) Mg concentration is low (below 1 4 mEq/l), even though serum Mg levels are much higher than normal (6.6 mEq/l compared with 2 1)

An objective of the present research was to confirm the contention that the Mg deficiency-induced hyperexcitability syndrome reflects the action of a central rather than peripheral mechanism This was attained by assessing the effects of cerebral intraventricular (CIV) Mg injections on Non-specific Excitability Level (NEL) and audiogenic seizure susceptibility A second objective was to determine the effects that these injections might have on brain neurotransmitters

There is scanty information published concerning this matter Itokawa, *et al* [11] found a marginal but not clearly significant decrease of serotonin (5HT) in the brains of rats fed a low Mg diet 25 days; however, the effect was not evident from later research [12]. Essman [8] found decreased brain 5HT levels in mice fed a low Mg diet 14 days.

To help clarify the 5HT issue and to determine if Mg status relates to other neurotransmitters, we analyzed the effects of CIV Mg injections on brain serotonin (5HT), norepinephrine (NE) and dopamine (DA). Intraventricular injections make possible the study of relationships between Mg status and neurotransmitter concentrations free of long-term dietary effects and complicating interactions involving peripheral and transport mechanisms

METHOD

Animal Care

Sprague-Dawley male rats were used in the three experiments reported They were housed individually in stainless steel cages and fed deionized water from plastic containers having stainless steel lick-spouts. Diet consisted of 58% dextrose, 20% casein, 10% corn oil, 2% vitamin mix [4], and 4% mineral mix [16] containing 0 75% calcium as carbonate, 0 6% phosphorus as monosodium phosphate and 0.27% potassium as chloride Cellulose (6%) was added to make up

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to 100% The low Mg diet was found by analysis to contain less than 10 mg Mg per kg. Control diet was made by adding 400 mg Mg per kg as $MgCO_3$ to the low Mg diet Ambient temperature in the animal room was 25°C and lights were on daily from 0800 to 2000 hr

Animal Preparation and Intraventricular Injections

For Experiments 1 and 3, permanent cerebral intraventricular (CIV) cannulations were done on 150–200 g approximately 6-week-old rats in a manner similar to that of Mabel, *et al* [15] Cannulae (15 mm long) were prepared from 22 ga disposable syringe needles with the heads clipped off, they had a small copper wire flange soldered 5–6 mm from the point The rat was anesthetized with pentabarbital and placed in a stereotaxic instrument The skull was bared, 5 small holes were drilled and 4 small anchor screws inserted The cannula was inserted through the fifth hole, located 1 3 mm lateral at the rostral edge of bregma. Normal saline, pumped through the cannula during insertion at 10 μl per min by a syringe pump attached via 0 584 mm polyethylene tubing, was pressure-monitored by a transducer. A drop in pressure indicated proper placement of the cannula in the lateral ventricle, usually 3 0–3 5 mm deep Dental acrylic was poured about the cannula, flange and anchor screws, and allowed to dry The skin was sutured over it An insect pin, covered with petroleum jelly, was inserted the full length of the cannula to stopper it and prevent clogging

Experiment 1

Permanently cannulated rats were fed a low Mg diet 21 days, assigned to five groups of 5 and given 10 min injections containing 0, 2, 6, 17 or 50 μg Mg in 80 μl of artificial cerebrospinal fluid (CSF) (Table 1) A sixth group was not injected Injections were given by a syringe pump at a rate of 8 μl per min, after the rat was lightly anesthetized with ether, the insect pin removed, and the lead tubing attached They were tested for Nonspecific Excitability Level (NEL) and audiogenic seizure susceptibility 10 min following the injections before much Mg could diffuse into the general circulation Testing was done between 1300 and 1700 hr Immediately thereafter, CSF was withdrawn from the cisterna magnum for analysis by atomic absorption spectroscopy

TABLE 1
COMPOSITION OF ARTIFICIAL CSF*

Approximate Ion Concentrations (mM/l)				μg Mg per 80 μl Injection (By Analysis)
MG^{++}	Na^{+}	K^{+}	Ca^{++}	
0	150	3	3	0
1	149	3	3	2
3	147	3	3	6
10	140	3	3	17
30	120	3	3	50

*All mixtures were made from chloride salts and 0.05% albumin buffer, and adjusted to pH 7 4 with dilute NaOH

Experiment 2

Acute cannulations were performed on weanling rats (50–60 g) after they were fed a low Mg diet 14 days Cannulae were inserted 1 mm lateral and 1 3 to 2 0 mm deep. These rats, assigned to two groups (N=10, N=9), were lightly anesthetized with ether and given 5 min injections of 0 or 25 μg Mg in 40 μl of artificial CSF NEL and audiogenic seizure susceptibility were assessed 15 min later, after the cannula was withdrawn and when the effects of anesthesia had worn off

Experiment 3

Sixteen permanently cannulated rats were fed a low Mg diet for 21 days Eight others were fed control diet Half of the rats from each group were injected with 2 μg Mg to provide a normal concentration in the CSF The other half of each group were injected with 50 μg Mg injections and NEL testing was done as in Experiment 1 General least squares analysis was used to identify significant treatment effects

Behavioral Testing

Nonspecific Excitability Level (NEL) and audiogenic seizure susceptibility were measured as described previously [4] NEL was measured by counting certain large movements—rearing, nose raises, locomotion, grooming responses and fecal excretions—made during two min in a small 11×22×23 cm high chamber Audiogenic seizure susceptibility was measured by assessing, on a scale of 0–5 (0=no seizure, 5=lethal seizure), the severity of seizures induced by exposing rats for 1 1/2 min to 115 decibels created by school bells inside a metal chamber

Monoamine Analysis

Rats used in Experiment 3 were killed within 5 min after behavioral testing by the near freezing method of Takahashi and Aprison [18] The brain was excised in a cold box (−5°C) and stored under liquid nitrogen Monoamine extraction was done at 0°C by a modification of the method of Shea and Aprison [17] Each brain was homogenized with 25 ml cold (15/85, v/v) 1 N formic acid/acetone for 1 5 min in a Virtis blender at 30,000 rpm After centrifugation at 5000×g for 10 min, the supernatant was removed. The pellet was resuspended in 10 ml of the formic acid/acetone mixture After re-centrifugation the supernatant was removed and combined with the other fraction Three 10 ml aliquots of the combined supernatants were removed, shaken for 10 min with 8/1 chloroform/heptane and centrifuged for 5 min The lipid phase was aspirated away One ml of the aqueous phase was transferred to another test tube and dried at 37°C under a stream of nitrogen The sample tube was tightly capped and stored at −35°C for up to 1 week.

The N_2 dried tissue extract was solubilized in 1 ml of 0 005 N HCl Five ml of 0.25 M pH 8 1 carbonate buffer were added, and the mixture was passed through a fritted funnel Four ml were placed on a double column apparatus as described by Karaswa, *et al* [13] The appropriate eluants were analyzed spectrofluorometrically by the procedures outlined by these authors [13] Internal standards, made with 1 part 0 005 N HCl and 5 parts 0 25 M carbonate buffer, and spikes were carried through the same steps as the samples

RESULTS

The results of 10 min injections of artificial CSF containing different amounts of Mg into the lateral ventricles of 5 groups of Mg deficient, permanently cannulated rats (Experiment 1), are shown in Fig 1 Results pertaining to a sixth group, not injected, are also shown

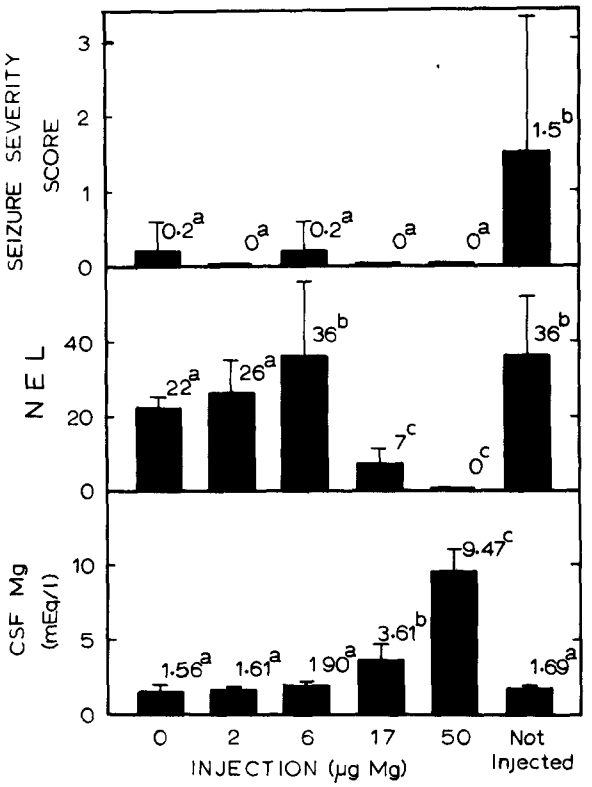

FIG 1 CIV Mg Injection-effects on CSF Mg Concentration, NEL and Audiogenic Seizure Susceptibility 150–200 g male rats were permanently cannulated and fed low Mg diet 21 days They weighed 217 ± 26 g on the final day Their serum Mg concentration was 0 78 ± 0 29 mEq/l and did not differ among groups There were 5 rats per injected group, 20 in the non-injected group [a]Values having different superscripts were significantly different ($p<0$ 05) Treatment differences were compared against least significant differences calculated from the pooled variances 0 399, 144 and 1 51 respectively, for CSF Mg concentration, NEL and Seizure Severity Score (40% of non-injected rats exhibited rapid running or more severe seizures)

Magnesium concentration of the CSF reflected the amount of Mg injected. NEL was decreased slightly with the 0 Mg injection, appeared to be normal when 6 μg of Mg was injected, and was decreased drastically with the larger doses Few of the injected animals seized

Because the above rats were apparently too old to seize consistently if injected, we sought to test CIV Mg injection-effects on younger animals However, since the skulls of younger animals grow rapidly, permanent cannulations were impractical Figure 2 shows the results of 5 min CIV injections of artificial CSF into the lateral ventricles of Mg deficient rats via acute cannulations (Experiment 2). The 25 μg Mg injection significantly lessened the seizure severity of these animals.

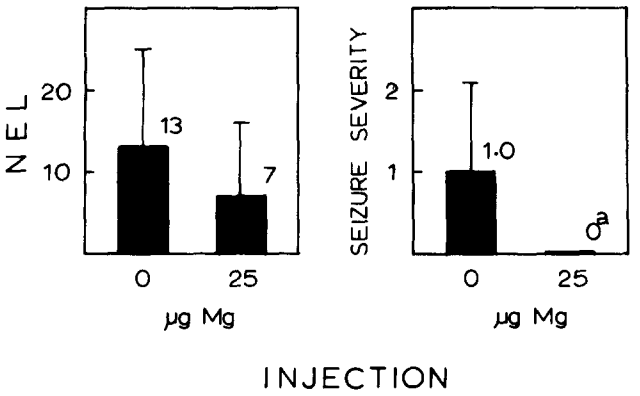

FIG 2 CIV Mg Injection-effects on NEL and Audiogenic Seizure Susceptibility Weanling rats were fed low Mg diet 14 days and were injected with 0 or 25 μg Mg via acute cannulations They weighed 91 ± 13 g on the final day N=10 and 9 for the 2 groups [a]This group had a significantly lower Seizure Severity Score than the 0 Mg injected group $t=2$ 67 ($p<0$ 02) (50% of the 0 Mg injected group exhibited rapid running or more severe seizures)

The effects of CIV Mg injections and a low Mg diet on NEL and brain monoamine concentrations in permanently cannulated rats (Experiment 3) are shown in Table 2 Compared with 2 μg injections, 50 μg Mg injections reduced NEL in both the low Mg and diet control groups 5HT concentration was greater in the brains of rats fed the low Mg diet than of dietary controls There was a significant DA interaction; the 50 μg Mg injected groups fed the low Mg diet had the most of this neurotransmitter Body and brain weights of rats fed the low Mg diet were not significantly lower than those fed control diet.

DISCUSSION

CIV injections of 17 μg Mg or more, compared with injections of 6 μg or less, decreased the excitability of our animals as assessed by NEL or audiogenic seizure severity (Figs. 1 and 2). This was observed while serum Mg concentration remained unchanged, confirming the hypothesis that the primary effect of Mg status on excitability is due to its action on the CNS and not upon the musculature. The only exception is when serum Mg concentration is high enough to produce a curare-like block [4]

The injection process alone reduces excitability (Fig 1), possibly due to increased ventricular pressure, a phenomenon known to have behavioral consequences [15] For this reason inclusion of sham-injected controls is necessary when undertaking CIV injection experiments.

There is an interesting qualitative difference between CIV Mg injection effects on NEL and audiogenic seizure severity. A 0 μg Mg injection reduces both NEL and seizure severity, however, a 6 μg Mg injection reverses this effect on NEL but not on seizure severity, and, all groups of rats receiving CIV injection displayed less seizure activity regardless of the Mg content. This loss of seizure activity due to the injection process forced us to use younger animals which are much more susceptible to dietary Mg deficiency-

TABLE 2

EFFECTS OF CIV MG INJECTIONS AND LOW MG DIET ON NEL AND BRAIN MONOAMINE CONCENTRATION (μg/g FRESH TISSUE) VALUES IN THE TABLE ARE GROUP MEANS N=8 FOR EACH LOW MG GROUP AND N=4 FOR EACH CONTROL GROUP AT EACH INJECTION LEVEL

Diet	Body Weight (g)	Brain Weight (g)	Parameter Measured	CIV Mg Injection 2 μg	50 μg
Low Mg	237 ± 26	1 69 ± 0 09	NEL	26 ± 12	0 9 ± 1 1*
			5HT (μg/g)	0 66 ± 0 07†	0 82 ± 0 23†
			NE (μg/g)	0 37 ± 0.03	0 39 ± 0 03
			DA (μg/g)	0 68 ± 0 10	0 79 ± 0 11‡
Control	263 ± 23	1 75 ± 0 08	NEL	35 ± 18	0 4 ± 0 3*
			5HT (μg/g)	0 53 ± 0 17†	0 58 ± 0 32†
			NE (μg/g)	0 35 ± 0 04	0 35 ± 0 05
			DA (μg/g)	0 73 ± 0 14	0 68 ± 0 04

*Significantly lower activity than that of the 2 μg MG injected groups, $F(1,20)=47\ 9$, $p<0\ 01$

†The main diet effect on 5HT was significant, $F(1,20)=7\ 38$, $p<0\ 02$ The injection effect was not clearly significant, $F(1,20)=2\ 64$

‡Significantly more DA than in brains of rats fed a deficient diet injected with 2 μg Mg and those fed control diet injected with 50 μg Mg, $F(1,20)=7\ 96$, $p<0\ 05$

induced hyperexcitability. Rats are consistently seizure prone only when CSF Mg falls significantly [4], a condition not met with the older animals However, even with the younger rats, only 50% were seizure prone, and the seizure severity score was low (Fig 2) This compares poorly with results from other experiments in which most of the (non-CIV injected) rats seized and the seizure severity score was high (4.1) [4,5]

The role of 5HT in moderating behavior is well established and has been reviewed [3, 6, 21] Brain 5HT levels bear inverse relationships to arousal and spontaneous activity Behavioral effects resulting from 5HT depletion include hyperactivity and seizure proneness Those accruing from increased 5HT levels include sedation and depressed activity In two recent reports, Green, *et al* [9] demonstrated an inhibitory effect of CIV 5HT injections on the spontaneous motor activity of rats, and Trimble, *et al* [20] showed that administration of 5-hydroxytryptophan (a serotonin precursor) to baboons completely blocked photogenic seizures

Based on our findings that brain 5HT concentration is increased in rats fed a low Mg diet, and the trend for CIV injections to increase 5HT, we conclude that 5HT may play a contributive role in mediating excitability Our results lay in the opposite direction of Essman's [8] who found that dietary Mg depletion reduces brain 5HT levels. However, his experiments were performed on younger subjects made acutely deficient in 16 days. From our activity data (Figs 1 and 2) [5], we observed that older rats fed a deficient diet longer than 14 days grow progressively less excitable, and their CSF Mg concentration is not drastically reduced Thus, if the arousal hypothesis applies, increased 5HT in the brains of the younger Mg deficient rats compared with controls may be expected, whereas it would not be expected in the brains of older animals such as used by us and Itokawa, *et al* [11] The fact that CIV Mg injections tended to increase 5HT, although not significantly (Table 2), lends further support to the possibility of a positive relationship existing between short-term manipulation of brain Mg and 5HT (and resultant behavioral excitability), while a negative relationship exists after several weeks. The increased 5HT in the brains of older animals fed a low Mg diet for the longer periods may be a protective adaptive response aimed at reducing the damaging effects of hyperexcitability It is possible that this is the same kind of compensatory response that leads to drug addiction Thus, initial administration of Mg may have a sedating effect which is compensated against over time Then if, after compensation occurs, Mg levels return to normal, hyperexcitability may ensue This, of course, has yet to be tested.

While the 5HT concentration response to CIV Mg injections may contribute towards reduced excitability, we cannot infer from our data that this is the only modulating effect There exists the possibility that magnesium ions activate 5HT receptors [19] Other mechanisms may operate. Magnesium is known, for example, to compete with a calcium mediated release of the neurotransmitter acetylcholine at the synapse of isolated preparations [10,14], it also reduces acetylcholine- and amino acid-induced excitation [7].

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