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Anticonvulsive Effect of *p*-Chlorophenylalanine in Mice on a Low Magnesium Diet

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ALEXANDER, G. J. AND L. M. KOPELOFF. *Anticonvulsive effect of p-chlorophenylalanine in mice on a low magnesium diet.* PHARMAC. BIOCHEM. BEHAV. 8(3) 291–293, 1978. – We have reported previously that para-chlorophenylalanine prevented seizure manifestations in an inbred strain of mice genetically predisposed to audiogenic seizures. We now find that this protective effect is not limited to a particular inbred strain of susceptible animals but can be demonstrated in Swiss-Webster and CF No. 1 strains which were not originally subject to audiogenic seizures but in which we have induced audiosusceptibility through maintenance on a low magnesium diet.

p–Chlorophenylalanine Audiogenic seizures Low-magnesium diet Serotonin Reflex epilepsy

WE have previously reported that audiosensitive inbred O'Grady mice were protected from audiogenic seizures following treatment with para-chlorophenylalanine (p–CPA) [4]. Among the mechanisms postulated for this action of p–CPA on audio-susceptibility was one related to a genetic predisposition, implying that the effect was limited solely to specific inbred animals strains.

In the present study, therefore, we have investigated the phenomenon in animals not genetically predisposed but rendered susceptible to sound-induced seizures by means of dietary magnesium deficiency.

METHOD

Two strains of non-audiosensitive mice were employed. One, derived from the Swiss-Webster strain, was bred in our own colony. The second, CF No. 1, was obtained from Carworth-Red Lion, New City, NY. In order to induce susceptibility to audiogenic seizures male weanlings of both strains, 10–15 g, were placed on a low-magnesium test diet containing less than 0.4 g Mg^{++}/kg, which was obtained from Nutritional Biochemicals Corporation, Cleveland, OH. Additional animals of each strain were maintained on a routine Teklad diet which contained 2.2 Mg^{++}/kg. Mice were kept in groups of 10 per cage and given food and water ad lib.

Para-chlorphenylalanine (p–CPA), obtained from Regis Chemical Co., Chicago, IL, was dispersed in 5% Tween–20 (Ruger Chemical Corporation, Yonkers, NY) and injected intraperitoneally (300 mg/kg). Control animals received a placebo of 5% Tween–20 vehicle (10 ml/kg). Injections were given between 11 AM and noon.

Susceptibility to audiogenic seizures was assayed as described before [1,2]. Individual animals were placed in a test chamber and exposed to an auditory signal of 22 kHz, 74 dBA produced by an RCA Signal Generator and projected through a high frequency speaker mounted on the roof of the chamber. The signal, set at a frequency inaudible to humans, was monitored by a sound level meter and presented for 30 sec. The severity of individual seizure responses was rated on a scale from 0 to 10 as described elsewhere [1]. Group score represented the sum of individual responses. Individual ratings of control and treated animals were used for statistical analysis. General level of activity of individual animals was measured with a Motility Tester obtained from Bel-Art, Pequannock, NJ.

In the first experiment, 20 Swiss-Webster mice were maintained on the magnesium-deficient diet. Motility, weight gain and developing susceptibility to audiogenic seizures were measured on the 8th, 11th, 14th, 21st and 28th day. Following the tests on the 28th day, 10 mice were injected with 300 mg p–CPA/kg and 10 with placebo. All were tested for seizure responsiveness at 2 hr and again at 24 and 96 hr and at one week. In the second experiment the same procedure was followed with mice of the CF No. 1 strain.

Additional mice of each strain which had been maintained on Teklad diet were tested similarly to determine whether any susceptibility to audiogenic seizures developed in the presence of normal concentrations of magnesium following treatment with either p–CPA of placebo.

RESULTS

Gross Behavioral Effects

Mice maintained on the magnesium-deficient diet became lethargic and showed low weight gain. After 4 weeks their weights averaged 27.0 ± 6.78 g compared to 39.7 ± 5.5 g in mice on the routine Teklad diet ($p<0.05$, Student's *t*-test). Motility of animals maintained on the low-magnesium diet decreased by approximately 50% (from 469 ± 151 to 238 ± 101 plate crossings in 30 min), while

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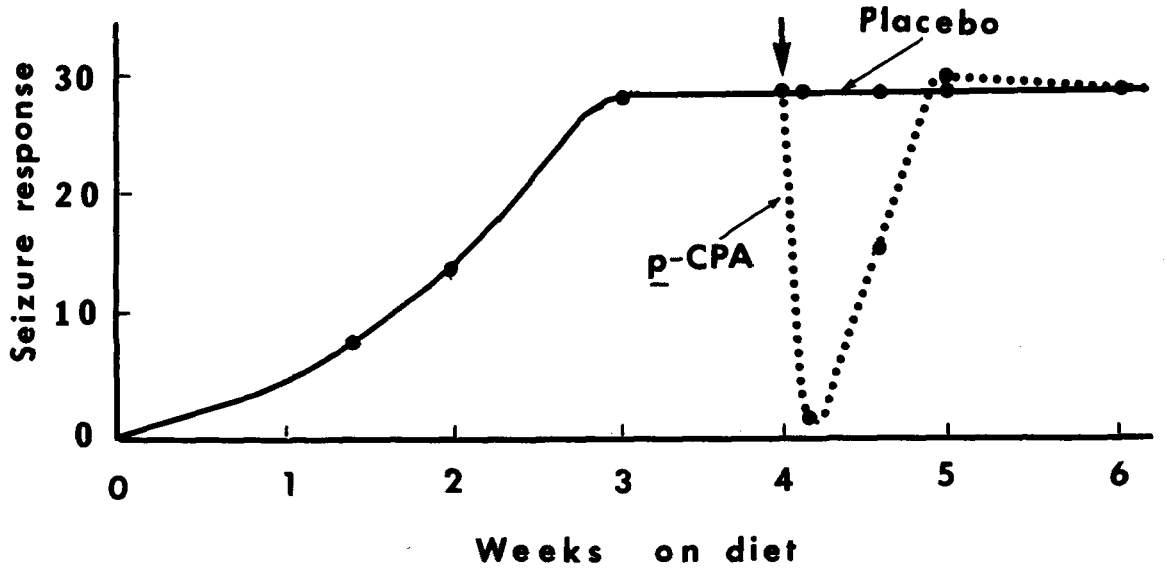

FIG. 1. Group seizure responses in CF No. 1 mice placed on a low magnesium diet and then treated with 300 mg/kg p–CPA or placebo (10 ml/kg). The group responses represent sums of responses of individual mice rated on a scale of 0–10 points [1].

motility of those on the Teklad diet remained unchanged. Treatment with p–CPA failed to rouse mice on the magnesium-deficient diet from their generally lethargic condition.

Maintenance on a low-magnesium diet led to the development of susceptibility to audiogenic seizures in 40–50% of the animals in 3–4 weeks. The reactions of the responders ranged from running only to running followed by clonic-tonic seizures with extension of the extremities. This group pattern corresponded closely to that observed routinely in our studies with inbred mice genetically predisposed to audiogenic seizures [1]. None of the mice which received the routine Teklad diet showed susceptibility to audiogenic seizures nor developed such susceptibility during the course of the experiments.

Effect of p–CPA on Susceptibility to Induced Audiogenic Seizures

Mice which had become audiosusceptible after 4 weeks on the low magnesium diet were protected from the epileptogenic effects of auditory stimulation by treatment with p–CPA. The maximum protective effect of a single dose of p–CPA in CF No. 1 mice were observed 24 hr after injection (Fig. 1). Prior to p–CPA treatment 4 of 10 mice responded to auditory stimuli with tonic-clonic seizures in 4–10 sec and one mouse with the uncontrollable running syndrome in 5 sec (Group seizure score: 29 points). One day later there was a complete absence of epileptiform responsiveness to the auditory stimulus. After one week full seizure susceptibility had returned. In the low-magnesium group injected with placebo, seizure susceptibility remained relatively unchanged, indicating that repetition of testing was not the significant factor.

Similarly, in the Swiss-Webster strain maintained on the low-magnesium diet maximum protection by p–CPA developed within 24 hr (Table 1). Seizure response in the p–CPA group decreased from the pre-treatment level (4 animals responding with tonic seizures and one with running) to complete absence of seizure manifestations in 24 hr. Response in the control group (low magnesium diet, treated with placebo) remained unchanged: 4 animals with tonic seizures in 5–9 sec.

TABLE 1

EFFECT OF P-CPA ON SUSPECTIBILITY OF SWISS-WEBSTER MICE MAINTAINED ON A LOW MAGNESIUM DIET TO AUDIOGENIC SEIZURES

	Positive Response		Group Score	
Time	p-CPA	Placebo	p-CPA	Placebo
0 hr	5/10	4/10	28.0	29.0
2 hr	2/10	4/10	11.0*	29.0
24 hr	0/10	4/10	0 *	30.0
96 hr	1/8†	4/10		28.0
1 week	2/8†	4/9†		

*$p<0.05$ (Student t-test)
†One or two animals died prior to this test.

Mice of both CF No. 1 and Swiss-Webster strains maintained on a low-magnesium diet showed the same response to p–CPA treatment: a gradual decrease in susceptibility to seizures induced by auditory stimulation from 4–5 per group prior to treatment to total remission at 24 hr with a return to pretreatment levels at one week. A comparison of seizure response ratings of individual control and p–CPA-treated animals showed that the decrease was statistically significant at the 5% level (Student t-test).

DISCUSSION

We have reported previously that treatment of inbred

audio-sensitive mice, O'Grady strain, with p-chlorophenylalanine (p–CPA) resulted after 2 hr in a decrease in susceptibility to audiogenic seizures [4,5]. This finding was in contrast to our own and other reports of increased susceptibility to various types of induced seizures 2–4 days after administration of p–CPA [3, 8, 9, 10]. The enhancement has been attributed to the inhibitory action of p–CPA on phenylalanine hydroxylase with the resultant selective depletion of brain serotonin. No definitive explanation is presently available for the anticonvulsive action of p–CPA which has up to now been demonstrated only in inbred mice genetically susceptible to audiogenic seizures. Our new data show that similar protection by p–CPA can be demonstrated in other experimental models, namely, non-inbred strains of mice rendered susceptible to audiogenic seizures by a diet low in magnesium. This new finding suggests that these additional models, in which the effects of inbreeding are eliminated, can be useful in studies of the role of biogenic amines in seizure control.

Dietary magnesium is known to influence seizure control mechanisms. Parenteral administration of large amounts of magnesium resulted in a marked depression of CNS activity, an effect reported useful clinically in the management of some convulsive manifestations [7], while hypomagnesemia induced neural hyperactivity and convulsions [11]. In rodents a low magnesium diet led to the development of susceptibility to audiogenic seizures, as described extensively in the literature and summarized by Bevan in 1955 [6]. We did not attempt to investigate the role of magnesium in seizures but used a commercially available low Mg diet to obtain non-inbred audiosusceptible animals for a study of the effects of p–CPA.

One significant difference between the otherwise comparable effects of p–CPA on seizures in mice on the low-Mg diet and the previously studied mice genetically predisposed to audiogenic seizures was the time of peak protection. The maximal effect of p–CPA occurred at 24 hr in the former but at 2 hr in the latter [4,5]. A study of the distribution pattern of serotonin in both brain and other sites in the two groups of animals at critical times following p–CPA administration is in progress. Correlations between changes in serotonin and protection from seizures may provide clues to the location of serotonin compartments involved in the control of susceptibility to audiogenic seizures.

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