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BRIEF COMMUNICATION

Effects of Phenylalanine and 5-Hydroxytryptophan on Seizure Severity in Mice

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TRUSCOTT, T. C. *Effects of phenylalanine and 5-hydroxytryptophan on seizure severity in mice.* PHARMAC. BIOCHEM. BEHAV. 3(5) 939–941, 1975. – The degree of audiogenic seizure was measured in DBA/2J (phenylalanine hydroxylase deficient) mice as a function of dietary phenylalanine (Phe) and injected 5-hydroxytryptophan (5-HTP), the precursor of serotonin (5-HT). Phe was shown to exacerbate seizures significantly, and seizure severity was found to be directly related to dietary concentration when animals were not treated with exogenous 5-HTP. 5-HTP was observed to significantly ameliorate seizures. The seizure-intensifying effect of Phe was reversible by 5-HTP injection and protection against seizures was directly related to 5-HTP concentration for animals on a high Phe diet. The results of this study indicate that Phe and 5-HTP are mutually antagonistic in modulating audiogenic seizure susceptibility.

Phenylalanine Serotonin Seizures 5-Hydroxytryptophan

THE exacerbating effects of phenylalanine (Phe) on seizure susceptibility in laboratory animals have been studied by using animals deficient in phenylalanine hydroxylase activity [11], by dietary Phe supplementation [5,13], and by Phe injections [4]. Para-chlorophenylalanine (PCPA), a Phe analog which depletes brain 5-hydroxytryptamine (5-HT), has been used along [10] or in conjunction with 5-hydroxytryptophan (5-HTP), the 5-HT precursor [3,12], to determine the effects of brain 5-HT manipulation on seizure susceptibility. These studies have generally shown that Phe supplementation and 5-HT depletion increase seizure susceptibility, while increasing brain 5-HT by 5-HTP administration ameliorates susceptibility to seizures. However, PCPA exhibits the dual action of inhibiting the metabolism of Phe and depleting 5-HT stores; it therefore seems to have limitations on its value for studying relationships between Phe and 5-HT with respect to their effects on seizure susceptibility. Recently, Boggan and Seiden [1] have demonstrated the reversing effects of 5-HTP on enhanced audiogenic seizure susceptibility following reserpine treatment.

The present study has utilized an experimental design in which both Phe and 5-HTP have been manipulated and the attendant susceptibility to audiogenic seizures measured. This experimental design permits assessment of both Phe and 5-HTP effects within the same experimental setting and additionally allows examination of any interacting effects of Phe and 5-HTP treatments. Since the DBA (dilute) strains of mice are particularly susceptible to audiogenic seizures [11] and have been shown to possess reduced Phe hydroxylase activity [2], they are an appropriate experimental animal for such studies.

METHOD

Animals

A total of 55 DBA/2J mice were used as experimental animals in a 3 X 3 factorial randomized group ANOVA design with unequal cell sizes of 5 to 8 mice per group. The origins and degree of inbreeding of these animals have been described by Jay [7]. The experimental animals were bred in the Animal Facility of the Albany Medical College from breeding stocks obtained from the Jackson Laboratory, Bar Harbor, Maine. All mice were maintained under standard conditions of temperature (26 ± 2°C) and lighting (12 hr light cycle) prior to use in any of the experiments, with ad lib access to water and the experimental diets. Mice of both sexes were used in approximately equal numbers. Animals were weaned at 20 days of age and tested at 22 days of age. All experiments were performed at the beginning of the dark portion of the normal light cycle, when DBA/2J mice have been shown to be maximally susceptible to audiogenic seizures [14].

Apparatus

The apparatus used for seizure testing consisted of a large glass chromatography jar (height 61 cm; dia. 29 cm)

with a 15 cm electric bell (Edwards) suspended 15 cm above the floor of the chamber to deliver approximately 115 decibels at the level of the mouse. Sound intensity was measured using a Type 410-A sound level meter manufactured by Hermon Hosmer Scott, Inc., Cambridge, Mass.

Procedure

For each experiment, mice were given varying amounts of L-Phenylalanine (L-Phe) by diet and L-5-hydroxytryptophan (L-5-HTP) by intraperitoneal injection. Each animal was removed from its cage and the injection procedure was carried out.

Phenylalanine. Three diets were used in the following experiments. Diet 1 was a normal laboratory diet of powdered Purina Commercial Mouse Chow (0.92 percent Phe in total ration, 9.06 percent Phe in amino acid composition by weight); Diet 2 was the same as Diet 1 but supplemented with approximately 1.0 percent L-Phe by weight (1.90 percent Phe in total ration and 17.22 percent of amino acids, by weight); Diet 3 was the same as Diet 1 but supplemented with approximately 2.0 percent L-Phe by weight (2.86 percent Phe in total ration and 24.3 percent of total amino acids, by weight). L-Phenylalanine was purchased from Nutritional Biochemical Corporation. The mothers of seven day old mice were placed on the respective experimental diets until the pups were weaned onto the diets at 20 days of age.

5-Hydroxytryptophan. For each experiment mice were given IP injections of either normal saline vehicle, 0.75 mg of L-5-HTP or 1.5 mg of L-5-HTP. The volume of each injection was 0.2 ml per mouse. L-5-Hydroxytryptophan was purchased from Nutritional Biochemical Corporation.

Seizure tests. Nine min after injection each animal was placed in the testing chamber and given 60 sec to adapt. The bell was sounded for 90 sec. During the testing period, the mice were observed for wild running, clonic, tonic and lethal seizures. Each incidence was given a seizure severity score according to the response: no response = 1; wild running = 2; wild running plus clonic seizures = 3; wild running, clonic, plus tonic seizures = 4; wild running, clonic, tonic and lethal seizures = 5. This scoring system was a modification of that used by Schlesinger, *et al.* [13].

RESULTS

Phenylalanine was found to enhance the severity of audiogenic seizures in DBA/2J mice, $F(2,46) = 10.39$, $p<0.001$. Figure 1 displays the mean seizure levels with their respective standard errors of the mean across levels of dietary Phe supplement for each level of injected 5-HTP. For all levels of 5-HTP dosage, the degree of seizure severity increased as dietary Phe concentration increased. The seizure-intensifying effect of dietary Phe was maximal and dose-related when no 5-HTP was administered and was only slight at a 5-HTP dosage of 1.50 mg.

A powerful protecting effect against audiogenic seizures was found for 5-HTP, $F(2,46) = 50.45$, $p<0.001$. No interaction effects were found for Phe and 5-HTP, nor were sex-related effects found. When data were reorganized across 5-HTP doses as in Fig. 2, 5-HTP was shown to reverse the Phe enhancement of audiogenic seizures. For animals not treated with supplementary Phe, 0.75 mg. of 5-HTP offered protection against audiogenic seizures by reducing seizure severity to nearly no response. Even at the high dietary Phe concentration (2.0 percent supplement), 5-HTP

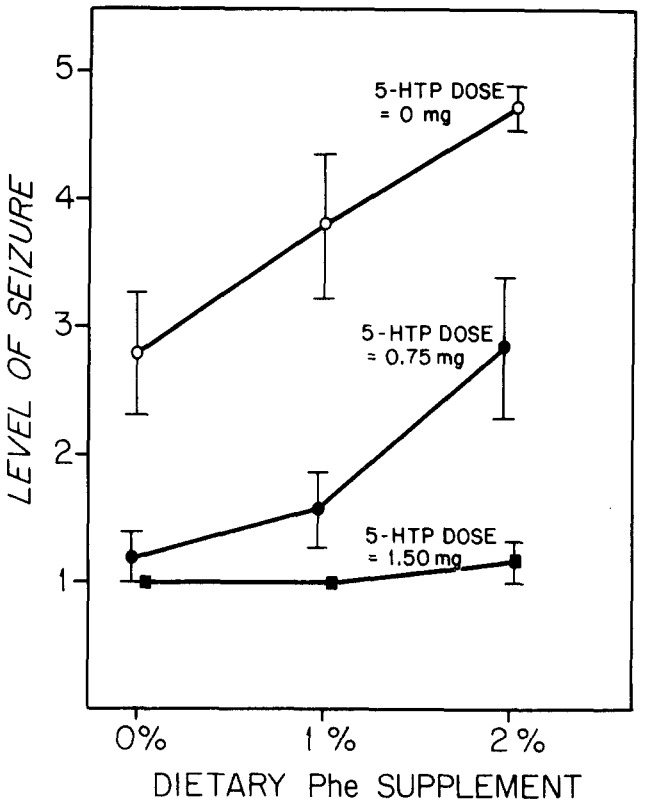

FIG. 1. Seizure severity ± one standard error of the mean across levels of dietary Phe supplement for each level of injected 5-HTP.

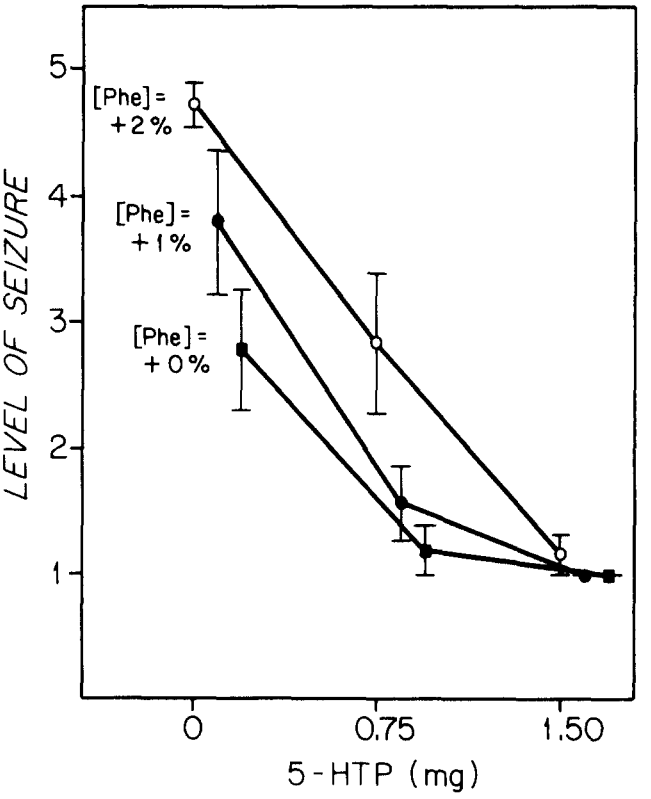

FIG. 2. Seizure severity ± one standard error of the mean across levels of injected 5-HTP for each level of dietary Phe supplement.

offered powerful protection against audiogenic seizures, with seizure level appearing to be inversely related to 5-HTP dosage. A nearly maximal effect was obtained with 0.75 mg of 5-HTP.

DISCUSSION

The results of the present study confirm previous work with regard to the seizure-enhancing effect of Phe in DBA/2J mice [13] and rats [5]. These results (Fig. 1) also agree with the report [4] that seizure level appears to be directly related to Phe dosage.

The present data support the findings [12] that 5-HTP offers a powerful protecting effect against seizures. This protecting effect furthermore appears to be linearly related to 5-HTP dose, at least for high Phe dietary treatment (Fig. 2). That 5-HTP is capable of reversing the seizure-enhancing effects of reserpine has previously been shown [1]. The present experiments demonstrate that 5-HTP is likewise capable of reversing the seizure-enhancing effects of Phe with the severity of seizure being determined by both Phe and 5-HTP dosage.

It has been reported that reduced brain 5-HT concentrations occur following high dietary levels of Phe [15]. Reversal by 5-HTP of reserpine-enhanced audiogenic seizures with concommitant 5-HT depletion appears to be dependent upon conversion of 5-HTP to 5-HT in brain [1]. However, inhibition by Phe of blood-brain barrier transport systems for tryptophan and other large neutral amino acids has been demonstrated [8]. It is possible that the 5-HTP treatments represent a by-pass of the Phe-inhibited reaction or transport system. Whether the mutual antagonism of 5-HTP and Phe is a manifestation of the Phe inhibition of the tryptophan or 5-HTP transport system into brain, inhibition of the rate-limiting tryptophan hydroxylase reaction [6], or a combination of the two effects [9] remains to be determined.

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