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Pentylenetetrazol-Induced Seizures in Pigeons and the Effects of Ethosuximide Thereon[1]

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JAKUBOW, J. J., H. SCHLINGER AND A. POLING. *Pentylenetetrazol-induced seizures in pigeons and the effects of ethosuximide thereon.* PHARMACOL BIOCHEM BEHAV **33**(1) 11–15, 1989.—Previous research has shown that ethosuximide in high enough doses disrupts operant responding in pigeons. Whether or not these same doses protect against seizure activity in this species has not been determined. In the present study a system for scoring pentylenetetrazol-induced seizures in pigeons was developed and the effects of ethosuximide on such seizures were evaluated. Pentylenetetrazol at 15, 27 and 47 mg/kg reliably induced seizures in Experiment 1. In Experiment 2 six doses of ethosuximide were tested for their seizure-controlling effectiveness. Doses of 20, 40, 80, 160 and 320 mg/kg ethosuximide had little effect on seizures induced by 27 mg/kg pentylenetetrazol; 640 mg/kg significantly reduced but did not completely eliminate seizures. This dose (640 mg/kg) is several times higher than the doses found to disrupt operant behavior in our previous studies.

Pentylenetetrazol Ethosuximide Seizures Pigeons

POLING and his colleagues have in the past five years examined the behavioral effects of several anticonvulsant drugs in pigeons (1, 3, 16–20). Results revealed quantitative and qualitative differences in the effects of various agents, but at high enough doses each drug tested interfered with operant behavior under one or more assay. Appropriate interpretation of these findings is problematic, for the relation of the doses that disrupted behavior to those that control seizures is unknown. It is possible that the doses that disrupted behavior in pigeons are far larger than those needed to control seizures. If so, these are simply toxic behavioral effects and of little interest.

To determine whether this is the case, dose-response curves for anticonvulsant activity in the pigeon must be compared to dose-response curves for behavioral activity. Generating the former curves requires a procedure for producing and evaluating seizure activity. Several procedures useful for generating seizures are available (2, 6, 9, 11, 12, 15). The one used in the present studies involved injections of pentylenetetrazol (PTZ).

Pentylenetetrazol reliably induces seizures in a variety of species and is useful in the study of anticonvulsants (5, 7, 10). Studies with chickens and pigeons have demonstrated that the drug produces changes in EEG activity and overt behavior indicative of seizures (2, 6, 8, 9, 11–14). In chickens, four behavioral changes accompany PTZ-induced convulsions as indexed by EEG activity (15). They are 1) convulsions, which involve (a) an initial excited state, (b) a tonic convulsion accompanied with opisthotonus and, (c) final convulsive movements; 2) peck-like behavior; 3) watchful behavior; and 4) panting. Our pilot studies with pigeons indicate that PTZ at nonlethal doses reliably induces rhythmic spasms that are most evident in the neck. Experiment 1 describes a system for scoring these spasms and presents a dose-response curve for PTZ-induced convulsions in pigeons. Experiment 2 reports the effects of ethosuximide (20–640 mg/kg) on these seizures. Ethosuximide (ESM) is a succinimide that controls PTZ-induced convulsions effectively in rodents (4,21). It is primarily used clinically to treat absence seizures, and is very effective at doing so (21).

EXPERIMENT 1: PENTYLENETETRAZOL-INDUCED SEIZURES

METHOD

Subjects

Twelve pharmacologically-naive White Carneaux pigeons maintained on ad lib food and water served as subjects. Subjects were individually housed in a constantly illuminated colony area.

Apparatus

The experimental chamber measured 61 cm long, 34 cm deep

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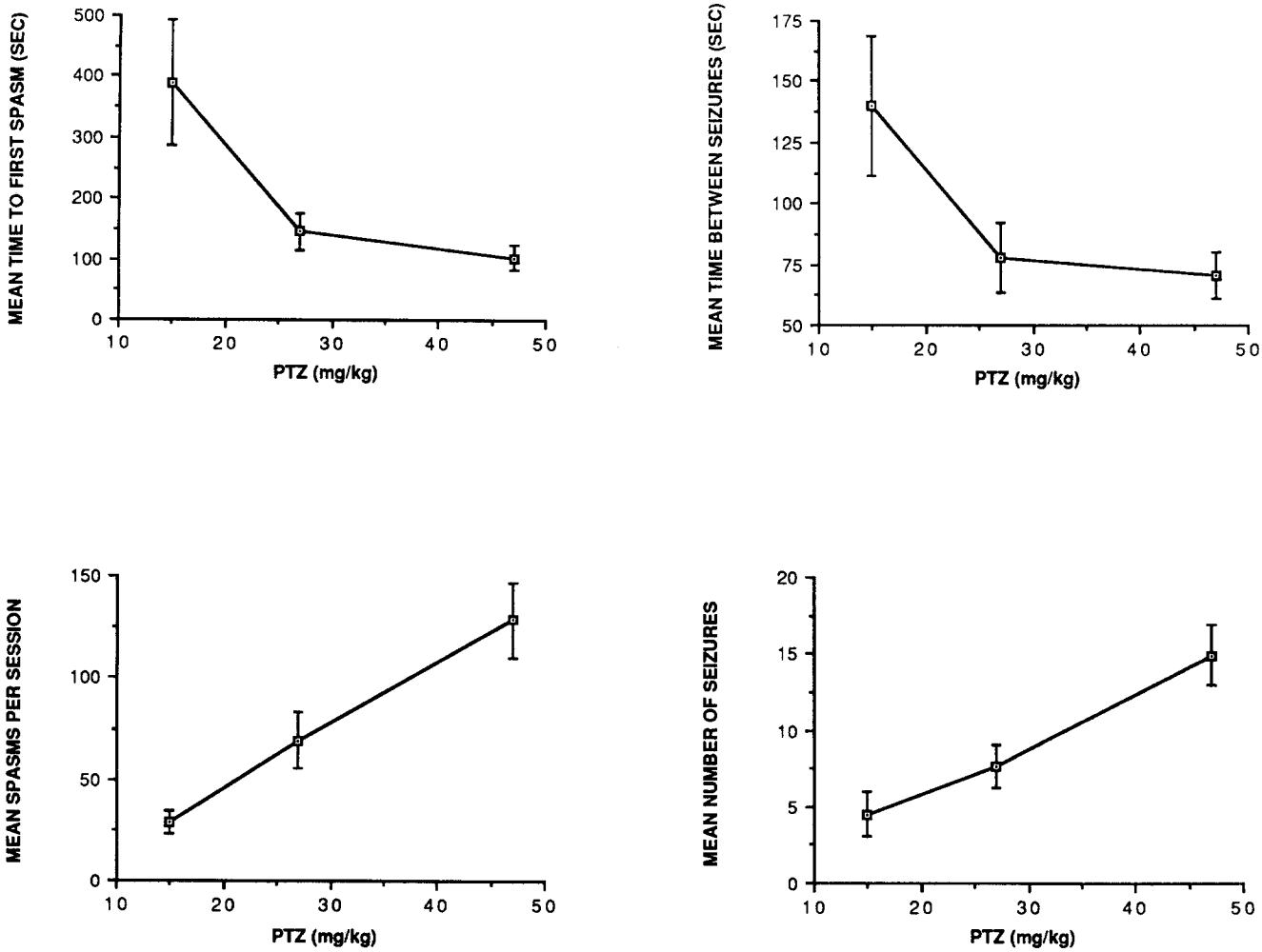

FIG. 1. The mean time to the first spasm, the mean time between seizures, the mean number of spasms that occurred in an experimental session, and the mean number of seizures that occurred in an experimental session for subjects given PTZ in Experiment 1. The measure of variability is ±1 SD.

and 46 cm high. Its floor, side walls and ceiling were made of plastic covered with carpet and the front was made of transparent plastic. One General Electric 25-W bulb inside a portable lamp was used for illumination. The lamp was located 61 cm in front of and 30 cm to the right of the top right corner of the experimental chamber. A video camera (Sony AVC-3200) stood 2.5 meters in front of the chamber on top of a tripod (Samson Quick-Set model 7201). A Panasonic Video Cassette Recorder (model NV-8200) and Sony Transistor Video Monitor (model CVM-112) were used to produce video recordings. Two STOPWATCH brand stopwatches (model PK-901) were used in timing dependent variable measures.

Procedure

Pentylenetetrazol doses of 15, 27 and 47 mg/kg were evaluated. The PTZ (Sigma) was dissolved in 0.9% sodium chloride solution, prepared at a volume of 1 ml/kg and injected intramuscularly (IM). Each bird was assigned a random ordering of drug doses and received each dose twice. Sessions were conducted every seven days. During each session PTZ was injected into the breast muscle, the bird was immediately placed in the chamber, and the video recorder was started. A session ended if no spasm was observed within 30 min or, if a spasm was observed, as soon as 5 consecutive minutes passed without a spasm. After every subject had been tested the video tape was played back and scored by two observers.

Spasm Definition

Pilot data indicated that nonlethal doses of PTZ induced spasms that were most evident in the neck. During spasms, the feathers of the neck were erect and the neck arched upward. This was followed by a downward movement of the head and neck that appeared concurrently with brief and rapid twitching of the body and/or wings. To allow ready measurement, a spasm was assumed to begin the instance that the head and neck lowered and the body began twitching and was considered ended when the head and neck rose to an upright position. Two or more spasms occurring without obvious temporal separation were labeled seizures.

Data were obtained by having two observers score the video tapes. The measures recorded were 1) time to the first spasm, 2) time between seizures, 3) spasms per session, and 4) seizures per session. To facilitate accurate measurement, the video recorder was played in slow motion until the observers agreed on when a spasm started and finished.

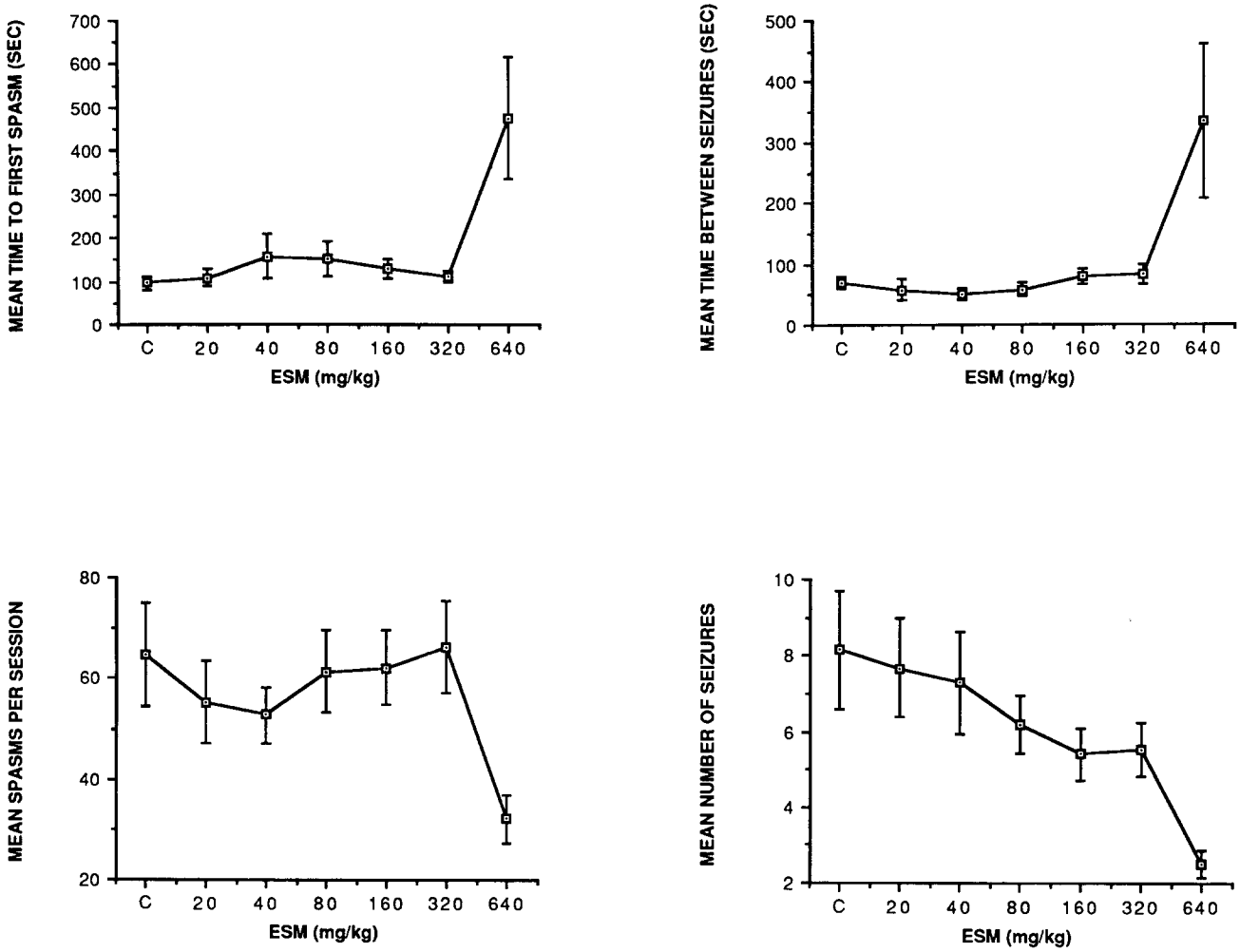

FIG. 2. The mean time to the first spasm, the mean time between seizures, the mean number of spasms that occurred in an experimental session, and the mean number of seizures that occurred in an experimental session for subjects in Experiment 2. Each bird received the listed dose of ESM 30 min prior to injection of 27 mg/kg PTZ. The measure of variability is ±1 SD.

RESULTS

Three birds died during Experiment 1. The data presented are from the nine subjects that completed the study. All data are represented graphically as group means (±1 SD), and were analyzed statistically by one-way repeated measures analysis of variance (ANOVA) followed by Tukey planned comparison tests. Results were considered statistically significant if the obtained alpha level was less than 0.05.

All data from Experiment 1 are shown in Fig. 1. All doses of PTZ induced seizures, although seizure activity increased with dose. The mean time to the first spasm varied significantly with PTZ dose, $F(2,34)=9.71$, $p<0.01$. Planned comparison tests showed that this measure was significantly higher at 15 mg/kg than at 27 mg/kg or 47 mg/kg. Increasing doses of PTZ also significantly reduced the length of time between seizures, $F(2,34)=6.14$, $p<0.01$. Planned comparison tests showed that this measure was significantly higher at 15 mg/kg than at 27 mg/kg or 47 mg/kg.

The mean number of seizures in an experimental session increased significantly with dose, $F(2,34)=14.34$, $p<0.01$. Planned comparison tests showed that the mean number of seizures was greater at 47 mg/kg than at 15 or 27 mg/kg. The mean number of spasms per session also increased significantly with PTZ dose, $F(2,34)=24.05$, $p<0.01$. Planned comparisons revealed that the increase in spasms from 15 to 27 mg/kg was significant, as was that from 27 to 47 mg/kg and from 15 to 47 mg/kg.

DISCUSSION

This experiment demonstrated that seizure activity increased with PTZ dose. The scoring system used appears to be adequate, insofar as the behavior of interest was easily quantified and varied lawfully with dose. Moreover, it was similar in topography to PTZ-induced seizures as previously described in chickens (15). Experiment 2 employed the general procedures used in Experiment 1 to evaluate the anticonvulsant effects of ESM.

EXPERIMENT 2: EFFECTS OF ETHOSUXIMIDE ON PENTYLENETETRAZOL-INDUCED SEIZURES

METHOD

Subjects and Apparatus

Subjects and apparatus from Experiment 1 were employed.

Subjects were maintained as in Experiment 1.

Procedure

Sessions were conducted as in the first study except that an ESM injection (20, 40, 80, 160, 320, or 640 mg/kg) was given IM 30 min prior to an IM injection of 27 mg/kg PTZ. Injections were given on opposite sides of the breast muscle. Each bird was assigned a different ordering of the ESM doses and the experimental sessions occurred once every seven days. Each subject received all ESM doses twice. Ethosuximide was obtained from Warner-Lambert (Ann Arbor, MI) and was dissolved in 0.9% sodium chloride solution. Pentylenetetrazol was prepared as described in Experiment 1. All drug doses were given at an injection volume of 1 ml/kg.

RESULTS

Data from this study were collected and analyzed as in Experiment 1 and are shown in Fig. 2. Statistical analysis revealed significant overall effects of ESM on mean time to the first spasm, $F(6,102) = 5.24$, $p<0.01$, mean time between seizures, $F(6,102) = 4.51$, $p<0.01$, mean spasms per session, $F(6,102) = 3.81$, $p<0.01$, and mean seizures per session, $F(6,102) = 4.75$, $p<0.01$. Planned comparisons revealed that for all measures the difference in means was significant ($p<0.05$) only between control and 640 mg/kg.

DISCUSSION

The effects of ESM (20–160 mg/kg) on the behavior of pigeons maintained under repeated acquisition, delayed-matching-to-sample, and multiple schedule procedures have been reported (16–18, 20). In those studies, doses of 80 and 160 mg/kg disrupted one or more aspects of operant behavior. Data from the present study (Experiment 2) indicate that these doses did not block PTZ-induced seizures; only the dose of 640 mg/kg did so. That dose produced severe behavioral impairment. Subjects receiving 640 mg/kg ESM lay on the floor of the cage with their eyes closed after approximately 10 min. When seizures did occur following injection of PTZ, the subject stood up, seizured, and then lay down again. In mice and rats, doses of ethosuximide far lower than 640 mg/kg provide protection against PTZ-induced seizures. The oral ED_{50} for protection against such seizures is approximately 193 mg/kg in mice and 54 mg/kg in rats (22).

An interesting finding of Experiment 2 was that subjects sometimes appeared to convulse before injection of ESM or PTZ. After the fourth week of testing, seizure-like behaviors were observed in all subjects when they were placed in their holding cages in the laboratory. These behaviors usually started approximately five minutes after the subject was placed in the holding cage and were topographically similar to the PTZ-induced seizures. They usually lasted about 10 seconds. Although this possibility was not evaluated systematically, it appears probable that these behaviors were respondently conditioned. Because the birds were always placed into holding cages before injection of a convulsant dose of PTZ, the holding cages perhaps came to act as conditional stimuli that eventually controlled behaviors similar to those elicited by PTZ. In some cases, subjects that did not show seizure-like behaviors in the holding cage did so immediately after the injection of ESM (regardless of dose), which implicated the injection itself or stimuli correlated with the injection as controlling the behaviors.

It is not known whether the seizure-like behaviors controlled by the holding cages and ESM injections were accompanied by changes in EEG activity indicative of actual seizures. In any case, these behaviors may have confounded both experiments. Because it is not known when the seizure-like behaviors began occurring and because they were indistinguishable from PTZ-induced seizures, one cannot differentiate in the data which seizure form was being scored on the video tape. This may have contributed to the relative lack of seizure-controlling ability of ESM. Some of the seizures recorded may have been respondently-conditioned seizure-like activity, not PTZ-induced seizures, and one would expect ESM to control only the latter. Thus it is possible, and perhaps likely, that the potency of ESM as an anticonvulsant in pigeons was underestimated in Experiment 2. Because of this, statements concerning the relative potency of ethosuximide as an anticonvulsant and as a disrupter of operant behavior must be made with caution. The dose of ethosuximide found to be anticonvulsant in the present study (640 mg/kg) was, however, several-fold greater than the doses that disrupted operant responding in our previous studies with pigeons. This suggests that ethosuximide at or below therapeutic doses may adversely affect behavior.

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