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A Study of Pentylenetetrazol Kindling in Rats and Mice

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CRAIG, C. R. AND B. K. COLASANTI. *A study of pentylenetetrazol kindling in rats and mice.* PHARMACOL BIOCHEM BEHAV 31(4) 867–870, 1988.—The effect of repeated injection of pentylenetetrazol on pentylenetetrazol seizure thresholds was determined in mice and rats. Once per week treatment of rats with pentylenetetrazol resulted in the development of a state of kindling. On the other hand, when pentylenetetrazol was administered twice per week, a phenomenon resembling tolerance was observed. In mice, it was not possible to demonstrate kindling under experimental conditions utilizing either one or two treatments with PTZ per week.

Pentylenetetrazol Kindling Tolerance

AN increased susceptibility of rats to pentylenetetrazol (PTZ) seizures after repeated injections of this drug was first observed in 1941 (13). At this time, it was demonstrated that the increased susceptibility to seizures was not lost even though months elapsed during which PTZ was not administered. This phenomenon has come to be called "pentylenetetrazol-kindling" or "Metrazol®-kindling" (11), as it shares many properties of a similar phenomenon in which repeated administration of an electrical stimulus results in a progressive intensification of seizure activity (3).

The primary objective of the present study was to characterize more fully the conditions necessary to induce pentylenetetrazol-kindling in the rat and to explore whether kindling can be demonstrated in the mouse. Early literature has suggested that mice living in aggregation may have lower PTZ seizure thresholds than those housed in isolation (15). This possibility was also further examined quantitatively in the present study.

METHOD

Seizure thresholds to PTZ were determined by a method previously documented (8). A seizure was considered to have occurred when a single episode of clonic spasms of both forelimbs and hindlimbs lasting at least 5 seconds and followed by loss of righting reflex was observed. In rats, PTZ, 15 mg/kg intraperitoneally (IP), was injected every 15 min until such a generalized motor seizure occurred (8). The thresholds were expressed as the number of 15-min periods to the onset of seizures. Six to 10 rats were used at each time point. Because higher doses of PTZ are required to produce seizures in mice (14), a dose of 20 mg/kg was given subcutaneously (SC) every 15 min until a generalized seizure occurred. In mice, at least 10 animals were used for each point.

The experimental populations were comprised of female Sprague-Dawley rats weighing between 150–200 g and either male or female Swiss-Webster mice, 20–25 g. Both rodent populations were purchased in their entirety prior to conduction of the studies and were maintained in the Animal Quarters except during test sessions. Animals were usually placed in individual viewing cages (4″×5″×7″) for the observation of seizures. In one series of experiments, however, mice were placed together in a larger cage (7″×11″) to determine any effects of aggregation on seizure thresholds.

Rats and mice were treated with PTZ either once or twice per week. As controls, one group of rats was tested initially with PTZ at week 1, and another group was first injected at week 8. In the case of mice, one group of animals was injected initially with PTZ at week 1, while a second group was given its initial injection at week 7.

Data were analyzed with the utilization of one-way analysis of variance (ANOVA) (Dyna-Stat®, Dynamic Microsystems, Inc., Pittsburgh, PA).

RESULTS

Figure 1 demonstrates the results obtained after once per week treatment of rats with PTZ. A significant reduction in the PTZ seizure threshold occurred by the third weekly administration of PTZ. Seizure thresholds remained depressed throughout the seven week period of the study.

On the other hand, when rats were treated twice per week with PTZ a significant reduction of seizure threshold was not apparent until week 4 (Fig. 2). By week 5, however, a significant elevation of the PTZ threshold occurred after the second weekly treatment.

In mice, either once (Fig. 3) or twice (Fig. 4) per week SC administration of PTZ produced a significant reduction in PTZ seizure threshold by week 2; the threshold remained depressed through week 7. However, as shown in Fig. 5, control mice which were included in the experiments but not treated until 7 weeks also showed a significant reduction in seizure threshold in comparison with values for mice treated

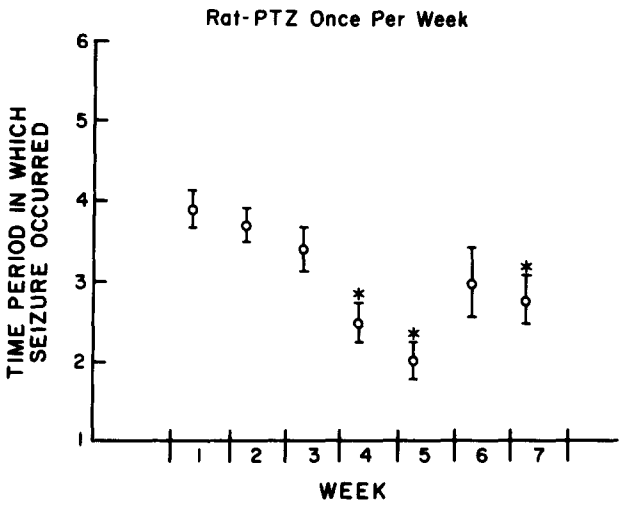

FIG. 1. Time period in which seizure occurred after once per week administration of pentylenetetrazol, 15 mg/kg IP every 15 minutes, to rats. Data are the mean±S.E.M. for 6 to 10 rats. *Indicates mean is significantly different from value at week one by ANOVA ($p<0.05$).

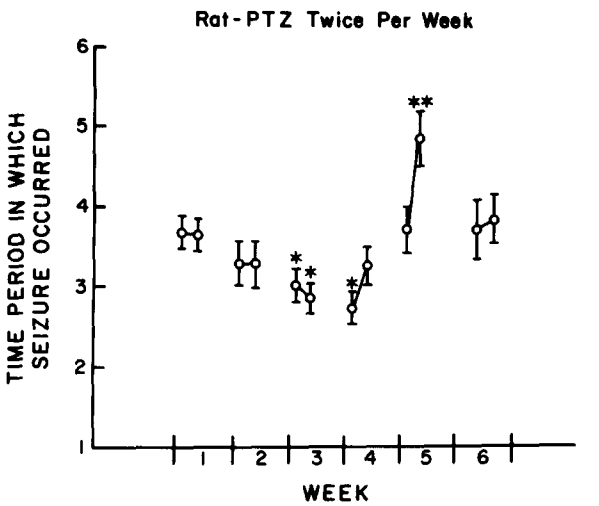

FIG. 2. Time period in which a seizure occurred after twice per week administration of pentylenetetrazol, 15 mg/kg IP every 15 minutes, to rats. Data are the mean±S.E.M. for 6 to 10 rats. *Indicates a significant reduction compared with the first treatment by ANOVA ($p<0.05$). **Indicates a significant elevation when compared with the first treatment by ANOVA ($p<0.05$).

at week 1. On the other hand, there was no significant difference between the PTZ seizure threshold of drug naive rats tested either 1 week or 8 weeks after the beginning of the study (Fig. 5).

As shown in Fig. 6, there were no significant differences between the PTZ thresholds of mice that were either isolated or aggregated during the experimental sessions.

DISCUSSION

Our results, which demonstrate a decrease in PTZ seizure threshold ("PTZ kindling") when rats are treated once per week, are in agreement with those of other investigators (1, 2, 13). Traditional electrical kindling in rats has become a widely employed technique for studying seizure mechanisms and is considered to be a useful experimental seizure model. Pentylenetetrazol-kindling represents a second means for studying a persistent decrease in seizure thresholds, although this method has been much less utilized.

An unexpected result of the present study was the apparent tolerance that developed at 5 weeks in the group of rats that was injected with PTZ twice weekly. This was not reported by previous investigators and may be due to differences in methodology. In the earlier reports, subjective grading of the severity of PTZ seizures from 0 to 5 (2) or 0 to 4 (1), rather than the time of seizure onset, was utilized as an end point. It should be noted, however, that Fig. 1 of (2) does show an apparent increase in seizure threshold after 5 daily injections of PTZ, although the authors made no comment on this occurrence (2). It may be postulated that the decreased seizure susceptibility observed in the present study 5 weeks after twice weekly injections of PTZ may be due to an induction of microsomal drug metabolizing enzymes, as PTZ is metabolized by the liver (7). Further studies are required to clarify this point.

Some earlier investigators have not been able to demonstrate PTZ kindling in rats (10). In the latter study, PTZ, 30 mg/kg IP, was injected daily for several days, after which the

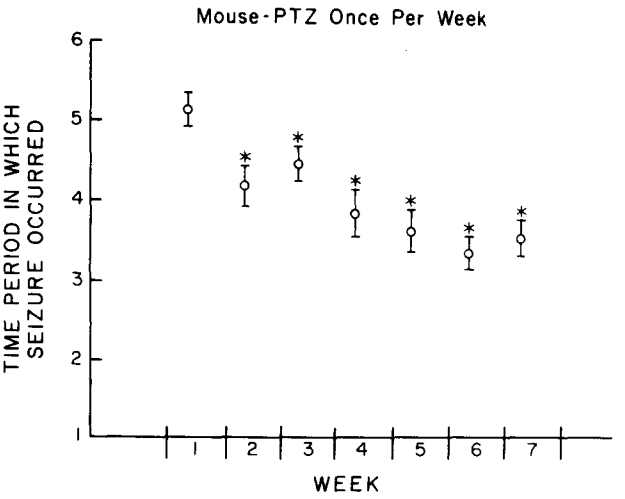

FIG. 3. Time period in which a seizure occurred after once weekly SC administration of pentylenetetrazol, 20 mg/kg every 15 minutes, to mice. Data are the mean±S.E.M. for 10 mice. *Indicates mean is significantly different from value of week one by ANOVA ($p<0.05$).

seizure threshold to IV PTZ was determined. The results indicated that there was no difference between the dose of infused PTZ necessary to elicit seizures in PTZ-treated rats and rats treated simultaneously with saline.

While our results did demonstrate PTZ kindling in rats, it was not possible to demonstrate such kindling in mice. Although the PTZ seizure threshold of PTZ-treated mice declined over time throughout the study, control drug-naive mice that were included in the experiments also showed a decreased seizure threshold when injected with PTZ at 7

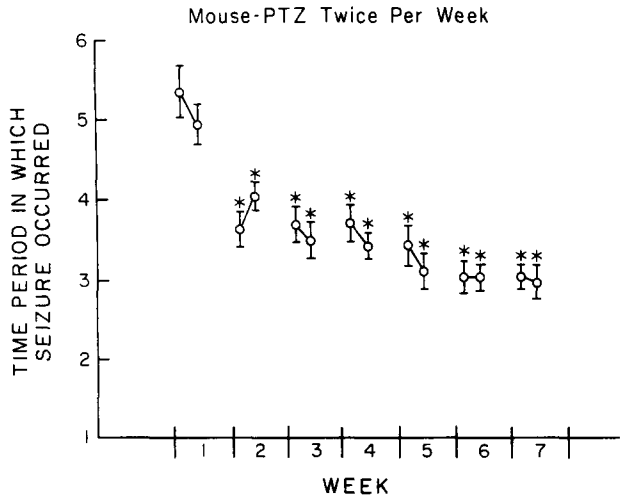

FIG. 4. Time period in which a seizure occurred after twice weekly SC administration of pentylenetetrazol, 20 mg/kg every 15 minutes, to mice. Data are the mean±S.E.M. for 10 to 12 mice. *Indicates mean is significantly different from value for the first treatment by ANOVA ($p<0.05$).

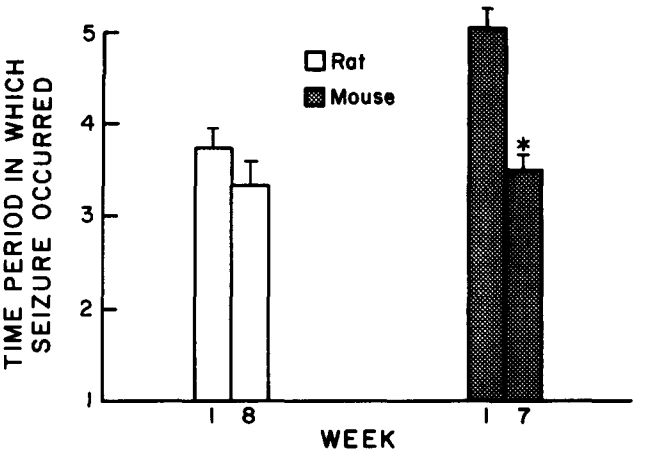

FIG. 5. Time period in which seizure occurred after pentylenetetrazol administration to drug-naive animals. Rats received pentylenetetrazol 15 mg/kg, IP every 15 min; one group (n=6) was injected at week one, while the second group (n=10) was not treated until 8 weeks later. Data are mean±S.E.M. Mice received pentylenetetrazol, 20 mg/kg, SC every 15 min. The first group (n=10) was tested at week one and a second group (n=10) was tested at week 7. *Indicates mean significantly different from value for first group by ANOVA ($p<0.05$).

weeks. A reason for this time- or age-dependent decrease in seizure threshold is not obvious.

The number of studies in which chemical kindling has been examined in mice is small. A recent paper reported results after repeated administration of pentylenetetrazol or picrotoxin in mice (5). In this study, repeated injection of either convulsant over a period of days did produce a lowering of seizure threshold, as seen in the present study in the case of PTZ. Likewise, an apparent kindling in 16 of 20 mice after 57 injections of pentylenetetrazol has also been reported (12). Still other investigators examined a beta-carboline benzodiazepine receptor ligand, FG7142, in similar studies and also showed an apparent kindling effect of this agent (9). None of the latter studies, however, utilized drug-naive mice in the experimental designs as controls. Therefore, it remains unresolved as to whether true kindling phenomena were observed in these earlier reports rather than a time-dependent nonspecific change in seizure threshold. To clearly establish whether PTZ kindling can occur in mice, a range of doses as well as several treatment regimens should be employed. The present experiments clearly indicate, however, that kindling is not a prominent feature in this species after chronic PTZ treatment.

A recent study has demonstrated age-dependent changes in the effectiveness of an anticonvulsant in mice (6). This report indicated that the minimal plasma concentration of phenytoin effective against maximal electroshock seizures was significantly lower in older mice than in their younger counterparts. In contrast with our study with the convulsant PTZ, wherein only 7 weeks were required to observe comparable findings, much longer time periods, e.g., 6, 12, 24 and 30 months were involved.

Certain centrally acting drugs exhibit marked changes in toxicity when experimental animals are grouped together rather than being housed in isolation. This is particularly apparent with some CNS stimulants such as amphetamine (4). In a study of the effect of ampehtamine on PTZ seizures

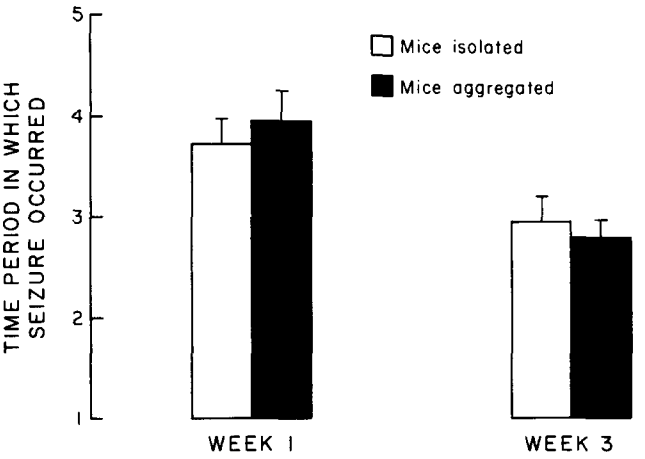

FIG. 6. The effect of isolation or aggregation on the time period in which seizure occurred after pentylenetetrazol administration (20 mg/kg, SC every 15 min) to mice. Data are mean±S.E.M. for 10–12 mice.

in isolated and aggregated mice (15) the authors presented data which showed that aggregation per se tended to lower PTZ seizure thresholds. Results of the present study fail to confirm these results. On the other hand, seizure thresholds of both the isolated and aggregated mice in our experiments did become lower over time, as observed for the remainder of the mice utilized throughout the present study.

ACKNOWLEDGEMENT

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REFERENCES

1. Albertson, T. E.; Peterson, S. L.; Stark, L. G. The anticonvulsant effects of diazepam and phenobarbital in prekindled and kindled seizures in rats. Neuropharmacology 20:597–603; 1981.
2. Diehl, R. G.; Smialowski, A.; Gotwo, T. Development and persistence of kindled seizures after repeated injections of pentylenetetrazol in rats and guinea pigs. Epilepsia 25:506–510; 1984.
3. Goddard, G. V.; Mcintyre, D. C.; Leech, C. K. A permanent change in brain function resulting from daily electrical stimulation. Exp. Neurol. 25:295–330; 1969.
4. Gunn, J. A.; Gurd, M. R. The action of some amines related to adrenaline. Cyclohexylalkylamines. J. Physiol. (Lond.) 97:453–470; 1940.
5. Karler, R.; Calder, L. D.; Sangdee, P.; Turkanis, S. A. Interaction between delta-9-tetrahydrocannabinol and kindling by electrical and chemical stimuli in mice. Neuropharmacology 23:1315–1320; 1984.
6. Kitani, K.; Musuda, Y.; Sato, K. Y.; Kanai, S.; Ohta, M.; Nokubo, M. Increased anticonvulsant effect of phenytoin in aging BDF1 mice. J. Pharmacol. Exp. Ther. 229:231–236; 1984.
7. Ko, G. K. W.; Hosein, E. A. The metabolic fate of pentylenetetrazol in the rat. Can. J. Physiol. Pharmacol. 49:356–365; 1971.
8. Levine, S.; Payan, H.; Strebel, R. Metrazol thresholds in experimental allergic encephalomyelitis. Proc. Soc. Exp. Biol. 113:901–902; 1963.
9. Little, H. J.; Nutt, D. J.; Taylor, S. C. Acute and chronic effects of benzodiazepine receptor ligand FG 7142: proconvulsant properties and kindling. Br. J. Pharmacol. 83:951–958; 1984.
10. Nutt, D. J.; Cowen, P. J.; Batts, C. C.; Grahame-Smith, D.; Green, A. R. Repeated administration of subconvulsant doses of GABA antagonist drugs I. Effect on seizure threshold (kindling). Psychopharmacology (Berlin) 76:84–87; 1982.
11. Pinel, J. P. J.; Van Oot, P. H. Generality of the kindling phenomenon: Some clinical implications. Can. J. Neurol. Sci. 2:467–475; 1975.
12. Piredda, S.; Yonekawa, W.; Whittingham, T. S.; Kupferberg, H. J. Enhanced bursting activity in the CA3 region of the mouse hippocampal slice without long-term potentiation in the dentate gyrus after systemic pentylenetetrazole kindling. Exp. Neurol. 94:659–669; 1986.
13. Sacks, J.; Glaser, N. M. Changes in susceptibility to the convulsant action of Metrazol. J. Pharmacol. Exp. Ther. 73:289–295; 1941.
14. Swinyard, E. A.; Brown, W. C.; Goodman, L. S. Comparative assays of antiepileptic drugs in mice and rats. J. Pharmacol. Exp. Ther. 106:319–330; 1952.
15. Swinyard, E. A.; Clark, L. D.; Miyahara, J. T.; Wolf, H. H. Studies on the mechanism of amphetamine toxicity in aggregated mice. J. Pharmacol. Exp. Ther. 132:97–102; 1961.