Neuropharmacology Vol. 28, No. 8, pp. 775–780, 1989
Printed in Great Britain

0028-3908/89 $3.00 + 0.00
Maxwell Pergamon Macmillan plc

PENTYLENETETRAZOL KINDLING IN MICE

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(*Accepted* 7 *December* 1988)

Summary—Kindling with pentylenetetrazol to produce minimal and maximal convulsions was investigated in CF-1 mice. Like electrical kindling, the kindling effect was directly proportional to the dose or the intensity of the kindling stimulus. Similarly, the kindling effect was persistent, as was emphasized by the ability to kindle with an interdose interval of 3 days and by the convulsions produced by a challenge with pentylenetetrazol 30 days after withdrawal from the kindling treatment. The changes in excitability, associated with the kindling state, appeared to be relatively selective for pentylenetetrazol, because no changes in thresholds to either electroshock or administration of picrotoxin or *N*-methyl-DL-aspartate correlated temporally with the persistence of kindling. The influence of two anticonvulsant drugs, ethosuximide and cannabidiol, on kindling was also investigated. Both drugs blocked the development of kindling to pentylenetetrazol-induced minimal convulsions. Of these drugs, only ethosuximide raised the minimal convulsive threshold to pentylenetetrazol. Against pentylenetetrazol-induced kindling to maximal convulsions, only cannabidiol blocked kindling and only cannabidiol raised the maximal seizure threshold for pentylenetetrazol. Although the drugs modified the kindling effect, the mechanism of the interaction is not clear.

Key words—pentylenetetrazol, kindling, anticonvulsants, cannabidiol, neurotransmitters.

Kindling, by definition, is an increased susceptibility to electrically-induced convulsions after repeated exposure to electrical stimuli (Goddard, 1967). The phenomenon has been described in many species (Racine, 1978), including the mouse (Sangdee, Turkanis and Karler, 1982). Chemical kindling also occurs and was first described for pentylenetetrazol by Mason and Cooper (1972) in rats; subsequently, there have been reports confirming the observation (see e.g. Pinel and Cheung, 1977; McCaughran and Manetto, 1982; Schmidt, 1987). The kindling phenomenon with pentylenetetrazol has also been reported in the mouse (Karler, Calder, Sangdee and Turkanis, 1984; Piredda, Yonekawa, Whittingham and Kupferberg, 1986). Little is understood about the mechanisms of kindling, or even whether electrical and chemical kindling share common mechanisms. This study provides a description of some of the characteristics of pentylenetetrazol-induced kindling in mice, especially as they relate to the characteristics of electrical kindling, and an evaluation of the influence of kindling on some neurotransmitter systems in the central nervous system (CNS). In addition, the results of studies of the effect of some anticonvulsants on the development of kindling are included.

METHODS

Male CF-1 mice, obtained from Charles River, were used in all experiments. They were initially about 6 weeks old and ranged in weight from 20 to 25 g. Minimal convulsions (forepaw and jaw clonus) and maximal convulsions (hind-limb extension) were selected as the endpoints for kindling. Kindling was produced by the repetitive subcutaneous injection of pentylenetetrazol which was administered in a dose regimen described for each experiment. Animals were given either 10 min to develop a minimal seizure or 15 min to produce a maximal seizure and any mouse that convulsed to the kindling treatment on day 1 was removed from the study. For the study of the persistence of kindling, animals kindled to minimal seizures were re-challenged with the kindling stimulus 15 and 30 days after withdrawal from the daily injections of pentylenetetrazol.

Mice, kindled to minimal seizures, were subjected to a variety of excitability tests of the CNS. Intravenously injected, pentylenetetrazol (1.35 mg/min), or picrotoxin (0.32 mg/min) or *N*-methyl-DL-aspartic acid (10.8 mg/min), was infused into the tail vein with a Harvard Apparatus infusion pump (Boston, Massachusetts), to obtain a mean convulsion threshold dose. The intravenous infusions were conducted in animals that were partially immobilized by the tail in a cylindrical Lucite holder (5 cm diameter and 8.5 cm deep). The Lucite cylinder had a slit in the top half to provide access to the tail and the size of the cylinder provided adequate room for an animal to exhibit convulsive behavior. The maximum convulsive threshold at 60 Hz, minimum convulsive threshold at 60 Hz and the minimal convulsive threshold at 6 Hz tests, were performed on kindled animals with the use of corneal electrodes (Karler and Turkanis, 1981); median convulsion thresholds were obtained using a step procedure (Finney, 1971).

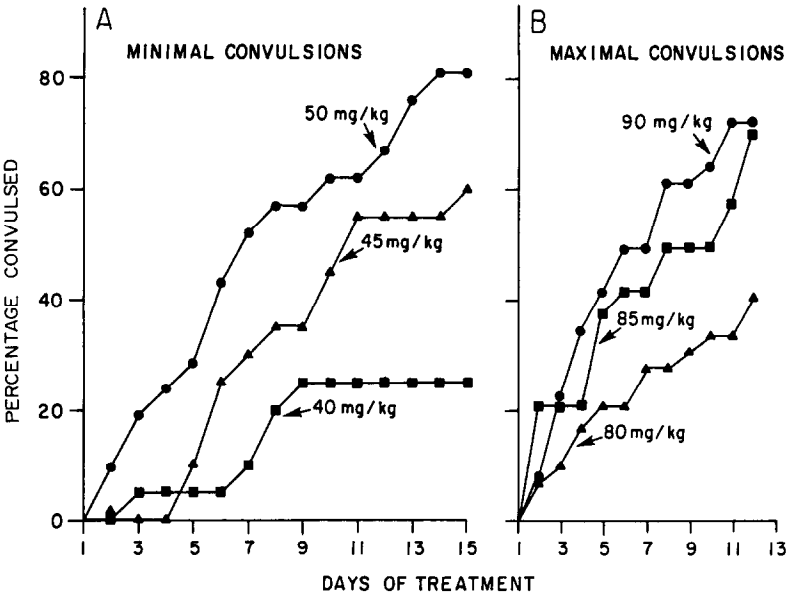

Fig. 1. Development of kindling to minimal or maximal convulsions, as a function of the dose of pentylenetetrazol. Six groups of 25–30 animals each, were given daily subcutaneous injections of pentylenetetrazol. As determined by a chi-square test ($P < 0.05$; Spiegel, 1961), curves obtained with 40 and 45 mg/kg were significantly different from that with 50 mg/kg and the curves with 80 and 85 mg/kg were significantly different from that with 90 mg/kg.

Anticonvulsant drugs were administered intraperitoneally prior to the injection of pentylenetetrazol; convulsive thresholds were measured at the times of peak-effect for the anticonvulsants i.e. ethosuximide, 30 min, and cannabidiol 1 hr. In the experiment where the endpoint was minimal seizures, the withdrawal of anticonvulsants was achieved by the removal of both the anticonvulsant and pentylenetetrazol for 15 days and then pentylenetetrazol was injected and convulsant activity was observed.

Pentylenetetrazol, picrotoxin and *N*-methyl-DL-aspartic acid were obtained from Sigma Chemical Co. (St Louis, Missouri), cannabidiol, from the National Institute on Drug Abuse, Washington, D.C. and ethosuximide, from Parke-Davis (Detroit, Michigan). Pentylenetetrazol, for subcutaneous injection, was dissolved in isotonic NaCl solution. Both cannabidiol and ethosuximide were prepared in a vehicle of isotonic saline with 3% Tween 80 (Turkanis, Cely, Olsen and Karler, 1974). The vehicle used for the intravenous injection of *N*-methyl-DL-aspartic acid, pentylenetetrazol and picrotoxin was isotonic saline with heparin, 10 units/ml of solution.

Results were plotted as the percentage convulsed on each day injected; percentages were cumulative so that mice which had kindled but died, due to the convulsion, were counted. A Mann–Whitney test was used to compare data from kindled and nonkindled animals (Siegel, 1956); a chi-square test was used to compare curves of the time-courses for the development of kindling (Spiegel, 1961). Median convulsion thresholds were compared by a relative potency test (Litchfield and Wilcoxon, 1949).

RESULTS

The data shown in Figure 1 represent the effect of different once-daily doses of pentylenetetrazol on the development of kindled convulsions. The data indicate that the development of either kindled minimal or maximal convulsions was directly proportional to the daily dose of pentylenetetrazol. Figure 2 shows the effect of varying the interdose interval on the development of kindled minimal and maximal convulsions. The data demonstrate that kindling occurred not only with once-a-day, but also with once-every-third-day treatment, although the kindling stimulus was more effective in the once-a-day regimen. The results for every-other-day treatment were also obtained but are not shown because they were similar to those for every day.

In a separate experiment, the persistence of the pentylenetetrazol-kindled state was examined directly. Separate groups of animals that were kindled to minimal convulsions were rechallenged with the kindling dose (45 mg/kg) 15 and 30 days after withdrawal of the drug. The results indicated that the kindled state persisted in all animals for at least 30 days, reflecting a relatively long-term change in excitability of the CNS. Because of the high mortality rate, associated with kindling to maximal convulsions, persistence studies were not done on this end-point.

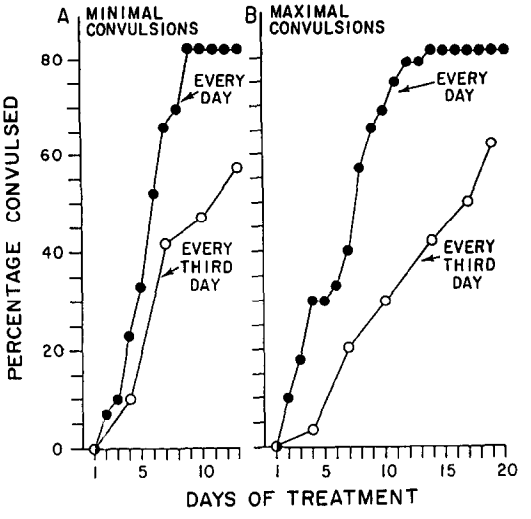

Fig. 2. Influence of the frequency of exposure to pentylenetetrazol on the development of minimal or maximal convulsions. Four groups of 30 mice each were given either 50 mg/kg (s.c.) for minimal, or 85 mg/kg (s.c.) for maximal kindling on a daily or every third day schedule. As determined by a chi-square test ($P < 0.05$; Spiegel, 1961), curves resulting from every-third-day treatment were significantly different from those of the daily treatment.

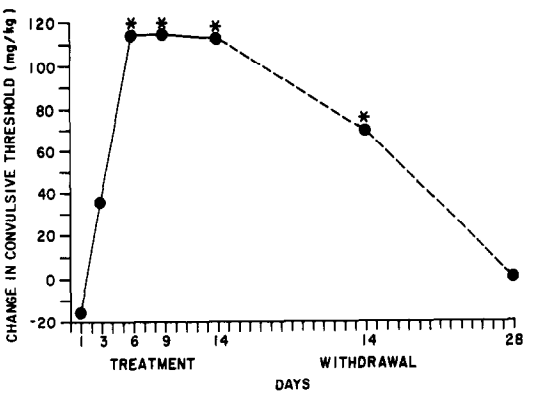

Fig. 3. Change in the maximal convulsive threshold to *N*-methyl-DL-aspartate during the development of kindling to pentylenetetrazol. Mice were treated once daily with pentylenetetrazol (90 mg/kg, s.c.) and, 24 hr after their last treatment, the thresholds were determined by the intravenous administration of *N*-methyl-DL-aspartate. Each data point represents the difference between the mean of a treated group and that of a control. All groups consisted of 10 mice. Asterisks indicate statistically significant changes ($P < 0.05$), as determined by a Mann–Whitney test (Siegel, 1956).

Table 1 represents data from an evaluation of the effect of kindling to minimal convulsions with pentylenetetrazol on excitability of the CNS, as determined by a variety of convulsive threshold tests with electroshock and chemicals. Besides the change in minimal threshold to pentylenetetrazol, only the thresholds to *N*-methyl-DL-aspartate were altered in the pentylenetetrazol-kindled mice; the thresholds for both minimal and maximal convulsions were elevated.

The data in Figure 3 show the results of the once-daily kindling treatment with pentylenetetrazol and withdrawal from treatment on the increase in maximal convulsive threshold for *N*-methyl-DL-aspartate as described in Table 1. By treatment day 6, there was a significant increase in the threshold for *N*-methyl-DL-aspartate and the increase persisted through to the end of the kindling period, day 14. During withdrawal, the thresholds were measured; by 28 days, they had returned to control level. Similar data were obtained for the minimal convulsive threshold for *N*-methyl-DL-aspartate.

The data in Figure 4 illustrate the effect of concomitant treatment with anticonvulsant drugs on the

Table 1. Convulsion thresholds of pentylenetetrazol-kindled mice

	Control	Kindled
Minimal convulsive thresholds:		
Pentylenetetrazol (mg/kg)	52 (45–61)	42 (36–49)*
N-methyl-DL-aspartate (mg/kg)	342 (258–368)	410 (314–507)*
Picrotoxin (mg/kg)	15 (13–19)	15 (12–18)
6 Hz (V)	13 (12–14)	15 (12–18)
60 Hz (mA)	5.5 (5–6)	5.5 (5–6)
Maximal convulsive thresholds:		
N-methyl-DL-aspartate (mg/kg)	490 (323–535)	604 (464–772)*
Pentylenetetrazol (mg/kg)	118 (98–148)	117 (101–139)
Picrotoxin (mg/kg)	23 (19–28)	24 (18–31)
60 Hz (mA)	9 (8–10)	9 (8–10)

Values from electroshock tests are median effective values and their 95% confidence limits and from tests with chemicals, the mean convulsion thresholds and their ranges. All values were obtained 24 hr after the last kindling treatment; 35–50 mice per electroshock value; 10–15 mice per value for chemically-induced convulsions. Animals were kindled to minimal convulsions by a once-daily subcutaneous treatment with pentylenetetrazol (50 mg/kg) for 14 days.

None of the median values was significantly different from control, as determined by a relative potency test ($P < 0.05$; Litchfield and Wilcoxon, 1949).

*Mean value was significantly different from control, as determined by a Mann–Whitney test ($P < 0.05$; Siegel, 1956).

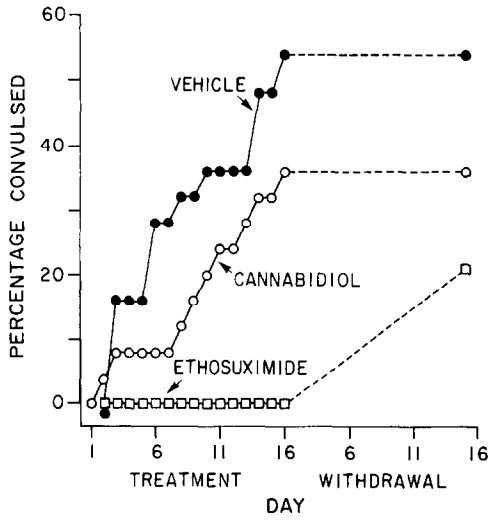

Fig. 4. Influence of treatment with anticonvulsant on the development of minimal convulsions to the daily administration of pentylenetetrazol. Three groups of 25–30 mice each were initially kindled with 45 mg/kg pentylenetetrazol in conjunction with daily treatment of either vehicle, 180 mg/kg cannabidiol or 200 mg/kg ethosuximide, given prior to the pentylenetetrazol. Animals were then withdrawn from both treatments for about 2 weeks and then retested for kindling with a single dose of 45 mg/kg pentylenetetrazol. As determined by a chi-square test ($P < 0.05$; Spiegel, 1961), the curves for cannabidiol and ethosuximide were significantly different from that for the vehicle control.

development of kindled minimal convulsions. As can be seen, both cannabidiol and ethosuximide retarded the development of kindling during the 16 days of treatment. In this experiment, after 16 days of daily anticonvulsant-pentylenetetrazol treatment, the animals were withdrawn for 14 days and then rechallenged with pentylenetetrazol. The extent of kindling in the controls and the group treated with cannabidiol remained unchanged after the withdrawal period, whereas about 20% of the previously unkindled animals treated with ethosuximide were kindled. These results suggest that pre-treatment with ethosuximide blocked not only the manifestation of motor convulsions but also the kindling process.

The influence of pre-treatment with cannabidiol on kindling with pentylenetetrazol to maximal convulsions is shown in Figure 5. As in the case of kindling to minimal convulsions, cannabidiol retarded the kindling.

DISCUSSION

Several previous studies of kindling with pentylenetetrazol in rats have been reported (e.g. Mason and Cooper, 1972), but there are relatively few studies of kindling with pentylenetetrazol in mice (Sangdee *et al.*, 1982; Karler *et al.*, 1984; Piredda *et al.*, 1986). The data presented represent a systematic description of kindling with pentylenetetrazol in mice, as well as a study of the effect of anticonvulsant drugs on the development of the kindling. The studies of the influence of dose and the interdose interval on the development of kindling and the persistence of the phenomenon were designed to compare these characteristics of kindling with pentylenetetrazol with those of electrical kindling in mice (Sangdee *et al.*, 1982). As with electrical kindling, the response was proportional to the intensity of the stimulus. As was also seen with electrical stimulation, kindling still occurred even if the interstimulus interval was 3 days. This suggests that the effect caused by exposure to the drug persisted for several days and was cumulative with repeated exposure. The persistence of the kindled state is also illustrated by the withdrawal data; that is, 30 days after withdrawal from the daily treatment with pentylenetetrazol, all of the animals remained kindled. Again, the persistence data resembled those for electrical kindling, which appeared to have a half-life of about 60 days in mice (Sangdee *et al.*, 1982).

As indicated, kindling with pentylenetetrazol in mice shared many of the characteristics previously described for electrical kindling. Nevertheless, there was a striking difference between the results described for these two methods of kindling; that is, with pentylenetetrazol, animals could be kindled to both minimal and maximal convulsions. In previous studies of electrical-induced kindling (Sangdee *et al.*, 1982), it was not possible to kindle mice to maximal

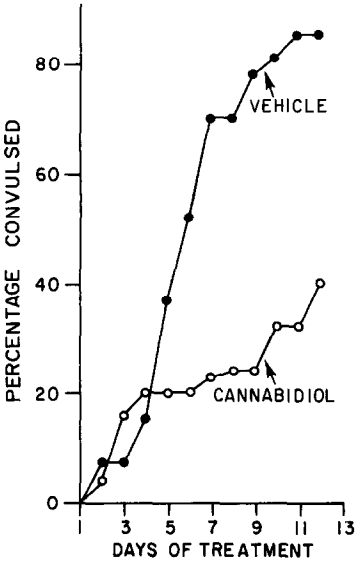

Fig. 5. Effect of the concomitant administration of cannabidiol on the manifestation of kindling to maximal convulsions, during daily treatment with 85 mg/kg pentylenetetrazol. Two groups of 25–30 mice each were given intraperitoneally either vehicle or 120 mg/kg cannabidiol prior to the daily subcutaneous injection of pentylenetetrazol. As determined by a chi-square test ($P < 0.05$; Spiegel, 1961), the cannabidiol curve was significantly different from that of the control.

convulsions. To date, electrically-kindled maximal convulsions in mice have not been described.

The thresholds for picrotoxin and *N*-methyl-DL-aspartate were determined as a means of assessing whether the increase in excitability, associated with the kindling, involved either a decrease in the functional properties of a major central inhibitory component, the γ-aminobutyric acid (GABA) system, or an increase in the functional properties of a major central excitatory component, the glutamate system. Neither effect was observed, which suggests that the increase in excitability to pentylenetetrazol was relatively selective. The observed decrease in responsiveness to *N*-methyl-DL-aspartate, however, may be linked to the development of kindling. Other investigators, for example, have reported that there is an increase in the release of glutamate in the brain, associated with an electrically kindled convulsion (Peterson, Collins and Bradford, 1983) and that in kindled animals, there is an increase in the brain in the concentration of *N*-acetyl-aspartyl-glutamate, a putative excitatory neurotransmitter (Meyerhoff, Koller, Walczak and Coyle, 1985). Furthermore, a glutamate antagonist has been shown to retard the development of kindling (Cain, Desborough, McKitrick, 1988). These data suggest that kindling involves enhanced activity of the glutamate system; such an effect could result in a down-regulation of glutamate receptors. The decreased responsiveness to *N*-methyl-DL-aspartate, described above, is consistent with a down-regulation during the development of kindling. The lack of persistence of the decrease in responsiveness to *N*-methyl-DL-aspartate, after withdrawal from the kindling treatment, however, did not correlate with the persistence of kindling, suggesting that the changes in responsiveness to the glutamate agonist, observed during the development of kindling, were not a component of the kindled state.

The studies of the effect of anticonvulsant drugs on kindling were designed to determine whether the development of kindling was retarded by drugs that increase the convulsion thresholds to pentylenetetrazol. Preliminary experiments involved ethosuximide and phenytoin: ethosuximide was used, because it increases minimal convulsive threshold to pentylenetetrazol (MacDonald and McLean, 1986), and phenytoin, because it increases maximal convulsive threshold to pentylenetetrazol (Turkanis *et al.*, 1974). After the initial experiments, phenytoin was dropped from the study because animals treated repeatedly with phenytoin and pentylenetetrazol died before kindling to maximal convulsions. For this reason, cannabidiol was substituted because, like phenytoin, it raises the maximal convulsive threshold to pentylenetetrazol, without affecting the minimal threshold; also it did not cause deaths during the kindling process.

The results demonstrate that ethosuximide, a drug that raises the convulsion threshold to minimal convulsions, retarded kindling to minimal convulsions; similarly, cannabidiol, a drug that increases the convulsion threshold to maximal convulsions, retarded kindling to maximal convulsions. The data show that cannabidiol blocked kindling to minimal convulsions even though the drug had no effect on the minimal convulsion threshold for pentylenetetrazol. These findings suggest that kindling can be blocked by a mechanism other than raising the convulsion threshold. More detailed studies of the influence of anticonvulsants on kindling may provide a better understanding of both kindling and anticonvulsant mechanisms.

Acknowledgement—This work was supported by NIDA research grant DA-00346.

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