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Effect of Pentylenetetrazol-Induced Convulsions on the Development and Expression of Limbic Kindled Seizures[1]

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GRAMLICH, C. A. AND J. S. STRIPLING. *Effect of pentylenetetrazol-induced convulsions on the development and expression of limbic kindled seizures.* PHARMACOL BIOCHEM BEHAV **26**(1) 159–165, 1987.—Male Long-Evans rats experienced three convulsions induced by intravenously administered pentylenetetrazol (PTZ) and were then kindled by electrical stimulation of the olfactory bulb or amygdala. Pretreatment with PTZ did not alter the rate of kindling in either site but did enhance the expression of kindled seizures once generalization had occurred (PTZ-treated animals had significantly longer motor seizures, measured by clonus duration, than did saline-treated controls). This suggests that PTZ-induced convulsions have selective effects on areas of the brain that are involved in the expression of the motor seizure. In addition, rats treated with PTZ after kindling had convulsions that were significantly longer in duration than any of their three pre-kindling convulsions, indicating that kindling produced an increased sensitivity to PTZ's convulsant effects. Comparison of this experiment with previous research suggests that the ability of a drug treatment to generate a kindling-like effect is related to the pattern of seizure activity that it produces.

Pentylenetetrazol Convulsions Kindling Olfactory bulb Amygdala Sensitization Rat

THE repetition of a stimulus can lead to an increased responsiveness to that stimulus, a phenomenon called sensitization. One form of sensitization is kindling, in which repeated electrical brain stimulation results in an intensification of the resulting afterdischarge, culminating in a generalized electrographic seizure that is accompanied by a behavioral convulsion [10,23]. Another form of sensitization occurs with the repeated administration of some convulsant drugs [7, 13, 31]. Pentylenetetrazol (PTZ) is such a drug and its repeated administration at subconvulsive doses (i.e., no generalized convulsion) can lead to an intensification of electrographic discharges and the development of a generalized motor seizure [7, 12, 15, 21]. This is sometimes referred to as PTZ kindling [1,19].

Electrical kindling and repeated PTZ administration can interact with each other. For example, rats are more responsive to PTZ after kindling [6,20], and pretreatment with PTZ can facilitate kindling development under some circumstances [7,30]. Cain [7] found that repeated intraperitoneal (IP) administration of subconvulsive doses of PTZ (29–32 injections) facilitated later kindling of the amygdala (AMYG), and Stripling and Russell [30] found that three convulsions induced by intravenous (IV) infusions of PTZ increased the rate of subsequent kindling in the neocortex. Stripling and Hendricks [29], however, found that three PTZ-induced convulsions did not affect the rate of kindling in the olfactory bulb (OB). These results [7, 29, 30] suggest that PTZ's effects on kindling may depend on the site of kindling.

This conclusion seems justified when comparing the neocortex and the OB because the same PTZ treatment facilitated kindling of the former but not the latter [29,30]. However, in those studies that found PTZ pretreatment to facilitate kindling in the AMYG [7] but not the OB [29], there were differences in patterns of PTZ-induced seizure activity and route and number of PTZ administrations as well as kindling site. This makes it difficult to evaluate the possibility that related limbic sites such as the AMYG and OB are differentially affected by PTZ treatment in regard to subsequent kindling. The present experiment was designed to assess this possibility. Rats were pretreated with physiological saline or three PTZ-induced convulsions and then kindled in either the AMYG or OB. If the different outcomes reported by Cain [7] and Stripling and Hendricks [29] were due to kindling site, then the PTZ treatment used here should facilitate kindling development in the AMYG but not the OB. Alternatively, if three PTZ-induced convulsions do not facilitate kindling at either site, then evaluation of the differences between this treatment and the one used by Cain [7] may provide insight into the nature of the kindling process.

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TABLE 1

DATA (MEANS ± S.E.M.) FOR PENTYLENETETRAZOL-INDUCED CONVULSIONS

	Convulsion 1	Convulsion 2	Convulsion 3	Convulsion 4
All Animals (n=28)				
Convulsive Threshold§ (mg/kg)	16.8 ± 0.5	19.3 ± 0.5*	21.1 ± 0.8†	—
Convulsion Duration (sec)	20.7 ± 0.7	20.2 ± 1.5	21.9 ± 1.9	—
Peak OB Amplitude (mV)	2.04 ± 0.12	2.42 ± 0.25	2.86 ± 0.23*	—
Peak AMYG Amplitude (mV)	2.30 ± 0.08	2.66 ± 0.13*	2.86 ± 0.13*	—
Animals That Were Convulsed Four Times With PTZ (n=5)				
Convulsive Threshold (mg/kg)	17.2 ± 1.1	19.6 ± 0.9	21.8 ± 1.3*	18.2 ± 1.6
Convulsion Duration (sec)	22.0 ± 2.5	19.7 ± 1.2	16.0 ± 0.9	69.8 ± 6.4‡
Peak OB Amplitude (mV)	2.00 ± 0.40	2.30 ± 0.40	2.30 ± 0.40	6.50 ± 1.10‡
Peak AMYG Amplitude (mV)	2.30 ± 0.40	2.50 ± 0.30	2.60 ± 0.30	4.90 ± 0.50‡

Specific comparisons using Tukey's (a) test ($p<0.05$):
*Significantly different from Convulsion 1.
†Significantly different from Convulsions 1 and 2.
‡Significantly different from Convulsions 1, 2 and 3.
§One rat received a subcutaneous PTZ infusion due to a faulty catheter and was excluded from this analysis.

METHOD

Subjects

The subjects were 66 male Long-Evans rats (Blue Spruce Farms) which weighed 260–410 g at surgery. They were housed individually and maintained on a 12 hr/12 hr light/dark cycle. Food and water were freely available.

Procedure

Animals were anesthetized with sodium pentobarbital (42.5 mg/kg) and chloral hydrate (100 mg/kg) and implanted with a Silastic catheter [28] in the right external jugular vein and two monopolar 200-μm diameter stainless-steel electrodes targeted for the left basolateral AMYG and the granule cell layer of the left OB. The coordinates for the OB electrode were 9.1 mm anterior to bregma, 1.2 mm lateral, and 1.5 mm below the dura. The AMYG electrode penetrated the dura 0.4 mm posterior to bregma and 4.0 mm lateral, and was lowered at an 8° lateral angle to a depth of 7.9 mm below the dura.

After a recovery period of at least 15 days, the animals were randomly divided into two groups and tested for afterdischarge (AD) threshold to electrical stimulation. One group (OB-KINDLED) was stimulated in the OB and the other group (AMYG-KINDLED) in the AMYG. The stimulation used for AD threshold determination and kindling was delivered by a Grass S48 stimulator and a PSIU6 stimulus isolation unit and consisted of a 2-sec train of 0.2-msec negative square-wave pulses at a frequency of 50 pulses/sec.

AD threshold was determined by repeated stimulation at 1-min intervals. OB-KINDLED animals were initially stimulated at 50 μA and the current was increased 50 μA/min until an AD was elicited. Because the amygdala has a lower AD threshold, AMYG-KINDLED animals were initially stimulated at 25 μA and the current was increased 25 μA/min. Rats not having an AD by 800 μA in the OB or 400 μA in the AMYG were excluded from the experiment. Five OB and three AMYG animals were excluded for this reason. The AD thresholds (mean±S.E.M.) were 441±28 μA in the OB and 145±10 μA in the AMYG.

Drug treatment began three days after AD threshold determination. Each group was divided into control and experimental subgroups matched for AD threshold. Animals in the experimental subgroups (n=15 each) were convulsed three times at three day intervals by IV infusions of 40 mg/ml PTZ. The drug solution was infused using a Harvard 975 infusion pump at a rate of 0.25 ml/min until the onset of a generalized convulsion. Animals in the control subgroups (n=14 each) received three saline infusions of comparable duration. Two rats died during the third PTZ-induced convulsion, leaving 14 animals in each group. Electrographic activity was recorded during each drug treatment using a field-effect transistor cable to minimize movement artifacts [25].

Eight days after the third PTZ-induced convulsion a second AD threshold was determined. Each animal was then kindled to criterion (2 consecutive days with clonus) by daily stimulation at 50 μA above its second AD threshold value. Any animal not exhibiting clonus within 30 ADs was excluded from the analysis of kindling. Each animal was to be convulsed with PTZ nine days after its last kindling stimulation, but this test was discontinued after three of the first nine rats to be treated died during the convulsion. Two of the three had been previously treated with PTZ, and the third with saline.

TABLE 2

DATA (MEANS ± S.E.M.) FOR THE DEVELOPMENT OF KINDLING AND THE EXPRESSION OF KINDLED SEIZURES

	OB-Kindled		AMYG-Kindled	
	Saline (n=14)	PTZ (n=14)	Saline (n=12)	PTZ (n=13)
ADs to Criterion				
2 Consecutive ADs With Clonus	9.9 ± 0.7	8.9 ± 0.8	15.6 ± 1.5*	18.9 ± 1.8*
First AD≥20 Sec	5.2 ± 0.4	4.3 ± 0.4	10.6 ± 1.6*	15.3 ± 1.8*
Expression of Kindled Seizures				
Number of Relapses in 5 Days of Clonus	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	1.4 ± 0.5*
Days of Clonus Before First Relapse (Ceiling=5)	4.9 ± 0.1	4.8 ± 0.2	4.7 ± 0.2	3.4 ± 0.4†

Specific comparisons using Tukey's (a) test ($p<0.05$):
*Significantly different from either OB-Kindled group.
†Significantly different from all other groups.

Histological examination of electrode placements indicated that all 56 OB electrodes were in or on the border of the granule cell layer. Thirty-seven AMYG electrodes were in the basolateral amygdala and the remainder were near the border of the AMYG and the pyriform cortex. The latter subjects kindled similarly to other animals and were not excluded.

Experimental effects were assessed by analysis of variance followed by specific comparisons using Tukey's (a) test. Variables exhibiting pronounced non-homogeneity of variance were subjected to logarithmic transformation prior to analysis.

RESULTS

PTZ-Induced Convulsions

Data for PTZ-induced convulsions are summarized in Table 1. A significant increase in convulsive threshold was found across the three PTZ-induced convulsions, $F(2,52)=18.64$, $p<0.001$, but convulsion duration did not change significantly, $F(2,54)=0.43$. Peak amplitude of the seizure discharges increased significantly across PTZ treatments in both the OB, $F(2,54)=6.07$, $p<0.01$, and the AMYG, $F(2,54)=12.85$, $p<0.001$.

Five animals that survived the post-kindling PTZ test (Convulsion 4) had received PTZ prior to kindling. For these five, there was a significant change in convulsive threshold across four convulsions, $F(3,12)=4.30$, $p<0.05$. A significantly larger dose was required to provoke a convulsion at Convulsion 3 than at Convulsion 1. There was also a significant change in convulsion duration across PTZ treatments, $F(3,12)=51.29$, $p<0.001$, with the duration of Convulsion 4 being significantly greater than any preceding convulsion. Peak electrographic seizure amplitude for these rats also changed significantly across PTZ treatments in both the OB, $F(3,12)=24.98$, $p<0.001$, and the AMYG, $F(3,12)=51.24$, $p<0.001$. Seizure amplitude was significantly larger during Convulsion 4 than during previous convulsions.

Kindling

Data for the kindling phase of the experiment are presented in Table 2. Three AMYG-KINDLED animals (2 treated with saline and 1 with PTZ) did not exhibit clonus within 30 ADs and were omitted from this phase of the analysis.

AMYG-KINDLED animals required significantly more ADs than OB-KINDLED animals to reach the kindling criterion of 2 consecutive ADs with clonus, $F(1,49)=47.24$, $p<0.001$. PTZ-induced convulsions did not significantly affect kindling rate, $F(1,49)=0.15$, nor was there a significant interaction between drug and kindling site, $F(1,49)=3.51$. To determine if PTZ-induced convulsions might have facilitated the development of electrographic, rather than behavioral, seizures, kindling was also analyzed to a criterion of 1 AD of 20 sec or more in duration. As before, AMYG-KINDLED animals kindled significantly more slowly, $F(1,49)=74.20$, $p<0.001$, and PTZ treatment was without significant effect, $F(1,49)=0.73$. However, there was a significant interaction between site and drug, $F(1,49)=6.49$, $p<0.05$. Inspection of Table 2 reveals that PTZ-treated animals performed slightly better than saline-treated animals in the OB group, but worse than saline-treated animals in the AMYG group, although neither of these differences reached significance by specific comparisons. These results indicate that the PTZ treatment used in the present experiment exhibited no tendency to facilitate the development of AMYG kindling to either a behavioral or electrographic criterion.

In order to determine if PTZ-induced convulsions influenced the expression of kindled seizures once they began to generalize, each animal continued to receive daily kindling stimulation until at least 5 ADs with clonus had been elicited. A 3-factor analysis of variance was then used to assess the effects of kindling site and drug on AD duration from the third AD preceding clonus to the fifth AD with clonus (see Fig. 1). AD duration was significantly longer in OB-KINDLED than AMYG-KINDLED animals, $F(1,49)=9.83$,

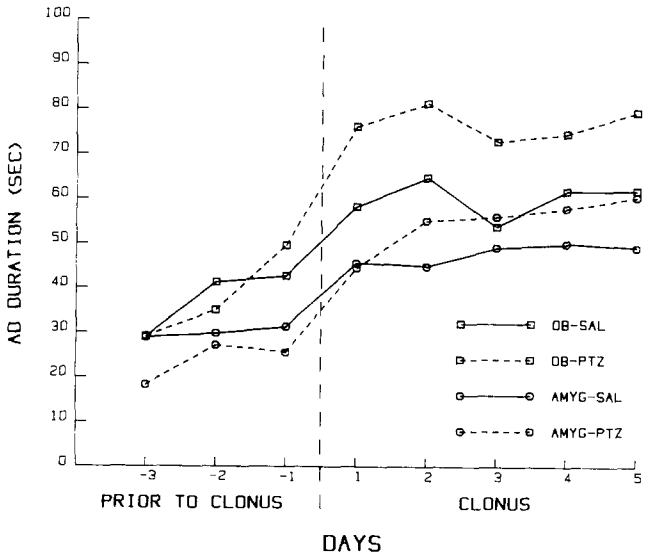

FIG. 1. Effect of kindling site and PTZ-induced convulsions on AD duration from the third day preceding clonus to the fifth day with clonus during kindling. The vertical dashed line marks the transition from ADs without clonus to ADs with clonus. OB=olfactory bulb; AMYG=amygdala; SAL=saline pretreatment; PTZ=PTZ pretreatment.

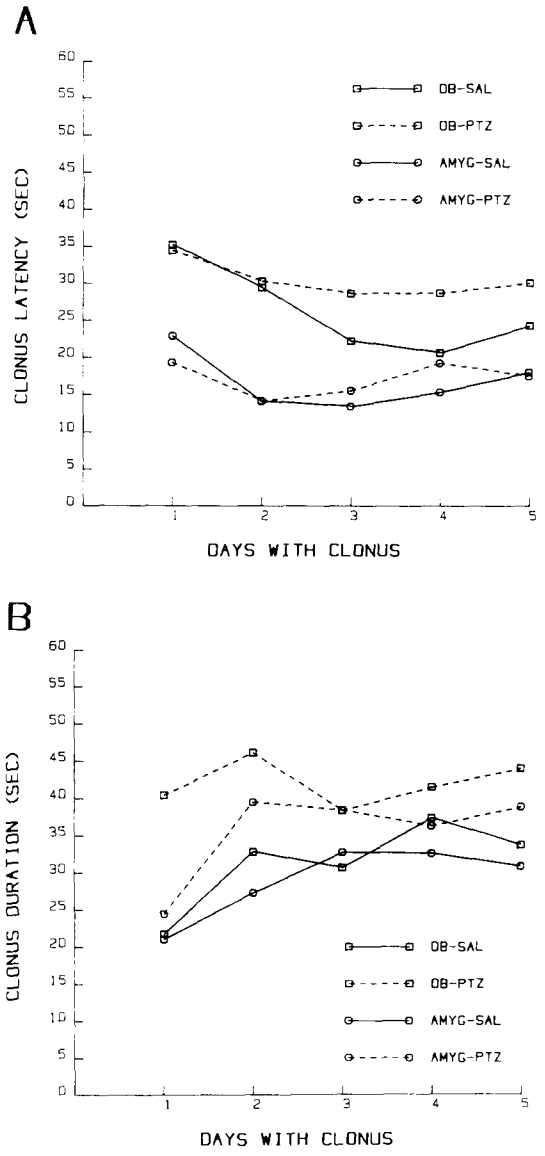

FIG. 2. Effect of kindling site and PTZ-induced convulsions on (A) clonus latency, and (B) clonus duration across the first five days with clonus during kindling. OB=olfactory bulb; AMYG=amygdala; SAL=saline pretreatment; PTZ=PTZ pretreatment.

$p<0.01$, and increased significantly across days, $F(7,343)=50.57$, $p<0.001$. As Fig. 1 indicates, this increase occurred primarily at the onset of clonus. There was a significant Site × Days interaction, $F(7,343)=2.08$, $p<0.05$, due to a greater increase among OB-KINDLED than AMYG-KINDLED animals, and a significant Drug × Days interaction, $F(7,343)=3.82$, $p<0.001$, due to a greater increase among PTZ-treated than saline-treated animals. No other effects were significant.

Expression of generalized seizures was examined more closely by dividing ADs with clonus into three components: clonus latency (the time required for the focal seizure to activate clonus), clonus duration (the generalized component of the seizure), and AD duration after clonus (persistence of the focal seizure after the end of clonus). Clonus latency (see Fig. 2A) was significantly longer in OB-KINDLED than AMYG-KINDLED animals, $F(1,49)=15.63$, $p<0.001$, and changed significantly over days, $F(4,196)=9.81$, $p<0.001$, with the latency on the first day of clonus being significantly longer than on any other. There was a significant Site × Days interaction, $F(4,196)=2.81$, $p<0.05$, due to a greater decline in OB-KINDLED than AMYG-KINDLED animals, and a significant Drug × Days interaction, $F(4,196)=2.62$, $p<0.05$, due to a greater decline in saline-treated than PTZ-treated animals. No other effects were significant. Because clonus latency was similar for saline-treated and PTZ-treated animals on the first day of clonus, the Drug × Days interaction did not appear to represent a direct effect of PTZ treatment on clonus latency; rather clonus latency exhibited less decline across days in PTZ-treated animals, perhaps due to a cumulative inhibitory effect of generalized seizures (see below). Clonus duration (see Fig. 2B) changed significantly over days, $F(4,196)=4.38$, $p<0.01$, with the duration on the first day of clonus being significantly shorter than on any other. In addition, clonus duration was significantly longer in PTZ-treated than saline-treated animals, $F(1,49)=4.98$, $p<0.05$. No other effects were significant. AD duration after clonus was brief and unaffected by any variable (largest $F=1.42$). Thus, of the three AD components examined, only clonus duration was significantly altered by PTZ-induced convulsions.

In the present experiment, some animals exhibited relapses in the expression of generalized seizures (i.e., failure to exhibit clonus during an AD after having exhibited it on a previous day). AMYG-KINDLED animals experienced significantly more relapses than OB-KINDLED animals in achieving 5 ADs with clonus, $F(1,49)=9.24$, $p<0.01$, and, although there was no significant drug effect, $F(1,49)=2.81$, there was a significant Site × Drug interaction, $F(1,49)=4.43$,

$p<0.05$. As Table 2 indicates, OB-KINDLED animals had a negligible number of relapses, while AMYG-KINDLED animals treated with PTZ had a significantly higher number than either OB-KINDLED group. The point at which generalized seizure expression became unreliable was assessed by analyzing the number of days an animal exhibited clonus before its first relapse, with 5 as an upper limit (see Table 2). There were significant effects of kindling site, $F(1,49)=8.94$, $p<0.01$, and drug, $F(1,49)=6.46$, $p<0.05$, and a significant Site × Drug interaction, $F(1,49)=5.17$, $p<0.05$. Specific comparisons indicated that AMYG-KINDLED animals treated with PTZ had significantly fewer days of reliable clonus expression before a relapse than any other group. However, even in this group relapses tended to occur towards the end of the 5 ADs with clonus. As a result, kindling to a criterion of 2 consecutive ADs with clonus proceeded reliably, with 52 out of 53 animals reaching this criterion without a relapse.

These data suggest that the relapses which occurred represent not an impaired ability to express generalized seizures but rather a proactive inhibitory effect of the first few generalized seizures on those that followed. The higher frequency of relapses among AMYG-KINDLED animals was probably due to the lower kindling current used: all animals were kindled at a current intensity 50 μA above their second AD threshold value, resulting in a kindling current of 124±6 μA for AMYG-KINDLED animals and 398±23 μA for OB-KINDLED animals. Among AMYG-KINDLED animals, those treated with PTZ were more susceptible to relapses and also had longer AD duration, which was due to longer clonus duration (i.e., the generalized portion of the seizure). This implies that those animals with the longest clonus duration during the first few generalized seizures should have the highest probability of a subsequent relapse. To assess this possibility, the summed clonus duration for the first two ADs with clonus was correlated with the number of days with clonus before the first relapse for each animal. A significant correlation was observed for both AMYG-KINDLED groups, $r(10)=-0.87$, $p<0.001$ for saline-treated animals, and $r(11)=-0.80$, $p<0.01$ for PTZ-treated animals, but not for the OB-KINDLED groups, $r(12)=0.11$ for saline-treated animals, and $r(12)=-0.27$ for PTZ-treated animals. These results indicate that long generalized seizures in AMYG-KINDLED animals were associated with an increased likelihood of a subsequent relapse. In contrast, at the stimulation current used in this experiment, expression of generalized seizures in OB-KINDLED animals was reliable regardless of the duration of previous generalized seizures.

DISCUSSION

Drug Effects on Kindling

The results of this experiment provide no clear evidence that PTZ-induced convulsions (a) produce sensitization to PTZ, (b) facilitate subsequent electrical kindling of limbic sites, or (c) differentially affect the OB and AMYG. However, these convulsions do enhance the expression of seizures previously kindled to the point of behavioral clonus. Full evaluation of these results requires that a clear distinction be made between the development of kindling in the limbic system and the expression of a previously kindled seizure. During limbic kindling, changes take place at the site of stimulation and elsewhere that allow seizure activity to spread progressively through the brain, eventually triggering a behavioral convulsion. Burnham [5] suggests that this convulsion results from the activation of core mechanisms in the brainstem and spinal cord. It might be expected that drugs which influence kindling development act at the seizure focus or at sites connecting the focus with the areas that produce the behavioral convulsion. This would allow seizure activity triggered by kindling stimulation to spread more rapidly. In contrast, drugs that influence only the expression of kindled seizures (i.e., motor seizures) might be expected to affect those areas directly involved in mediating or regulating the motor seizure. In this experiment, the expression of kindled seizures was affected by the PTZ pretreatment used, but kindling development was not. PTZ-treated animals exhibited longer AD durations only when ADs were accompanied by clonus (see Fig. 1). Of the three components of the kindled seizure, neither clonus latency nor AD duration after clonus was altered by PTZ pretreatment, but the motor seizure itself (clonus duration) was lengthened. Taken together, these results indicate that PTZ-induced convulsions selectively enhance the expression of motor seizures, perhaps by altering brainstem and spinal mechanisms involved in their production [5].

Other researchers have reported similar but transitory effects following administration of a subconvulsive dose of PTZ [2–4]. Bowyer and Albertson [3], for example, found that 25 mg/kg PTZ (IP) increased AD duration and the expression of motor seizures when given 15 min before each electrical stimulation during AMYG kindling. However, kindling performance returned to control levels in the absence of PTZ. Thus, PTZ did not actually change the rate of kindling development but produced a temporary increase in seizure expression. To examine drug effects on the expression of seizures kindled in the neocortex (area 6), Stripling and Russell [30] injected kindled rats with subconvulsive IP doses of cocaine (20 mg/kg), lidocaine (20 mg/kg), or PTZ (12 mg/kg) and then elicited an AD. The two local anesthetics had no significant effect on AD duration, but PTZ produced a significant increase, indicating that it was affecting seizure expression. Interestingly, Stripling and Russell [30] also found that three PTZ-induced convulsions facilitated the development of subsequent kindling in area 6 of the neocortex. Area 6 is a motor area and the rats typically exhibited clonus on the first stimulation [30]. The ability of PTZ-induced convulsions to facilitate kindling at this site may involve the drug's apparent ability to facilitate the expression of motor seizures.

The effects of local anesthetics such as cocaine or lidocaine on kindling are distinctly different from those of PTZ. Acute administration of local anesthetics in limbic-kindled animals selectively enhances the propagation of seizure activity from the site of stimulation [14], speeding the onset of the motor seizure without increasing its duration [26]. Convulsions induced by cocaine or lidocaine facilitate subsequent kindling of the OB [29] or AMYG [13] and facilitate the development of the electrographic but not the motor seizure in cortical kindling [30]. These results suggest that local anesthetics act most prominently on kindling development (especially the propagation of ADs from the site of stimulation) rather than on motor seizure expression.

Comparison of the present experiment with previous research provides some insight into the mechanism by which drugs produce sensitization and cross-sensitization with kindling. Cain [7] found that 29–32 IP injections of an initially subconvulsive dose of PTZ produced sensitization to the drug and facilitation of subsequent kindling of the AMYG.

The present experiment found neither of these effects following 3 generalized convulsions induced by IV infusions of PTZ. Two previous experiments in our laboratory have also found no sensitization to PTZ produced by 3 PTZ-induced convulsions [29,30]. Because both Cain [7] and the present experiment used the same site of stimulation for kindling, the discrepancy in these results appears due to differences in the drug treatment used. Such differences include route of drug administration, number and timing of drug treatments, and type of seizure activity produced by the drug. There is some evidence to suggest that drug sensitization is more easily established or expressed using IP rather than IV administration ([17]; see [30] for further discussion). However, Cain [8] demonstrated sensitization to PTZ using direct intracranial infusions, indicating that sensitization is at least in part a central phenomenon unrelated to route of administration. Although the number of drug treatments is likely to influence the size of the effect produced, we have found facilitation of limbic kindling following either one or three convulsions induced by cocaine but not by PTZ [29], indicating that the number of treatments is not the crucial issue.

The most important factor in determining whether a drug treatment will produce kindling may be the pattern of seizure activity produced by the treatment. Electrical kindling involves the stimulation of a specific brain site, triggering a focal seizure which spreads progressively across trials to other brain areas. Intracranial drug administration also creates a focal seizure which can result in sensitization to the drug and facilitation of kindling [8,9]. In contrast, generalized seizures such as fully kindled seizures or those produced by electroconvulsive shock (ECS) inhibit kindling [11,22] and seizure expression [11, 16, 22, 27] (see also the present study). Furthermore, ECS-induced seizures typically do not produce a kindling-like effect except at long inter-seizure intervals [24]. These observations imply that kindling is most readily produced by focal seizures, while strong generalized seizures can retard the process. In this regard it is interesting to note that the PTZ treatment we employed produced sustained generalized seizures, while that employed by Cain [7] did not, but rather resulted in the gradual emergence of twitching and myoclonic jerks accompanied by isolated epileptiform spikes. This suggests that drug treatments that produce seizure activity which is too prolonged, intense, or widespread may interfere with the development of a kindling-like process. Such interference might be related to the activation of seizure-inhibiting mechanisms by generalized seizures [16,18]. If so, PTZ-induced convulsions might produce a kindling effect if spaced further apart.

It is possible to extend this concept to differentiate between various types of generalized seizures. Burnham [5] proposes that the intensity of activation of core seizure mechanisms is reflected in the type of behavioral seizure produced, such that weak generalized seizures are manifested by forelimb clonus, and increasingly strong activation incorporates first hindlimb clonus, then forelimb tonus, and finally hindlimb tonus into the behavioral convulsion. Our laboratory has studied the effects of two types of generalized drug-induced seizures on kindling. PTZ induces intense tonic-clonic seizures which are indicative of a near-maximal convulsion. Small numbers (1–3) of these seizures do not facilitate subsequent limbic kindling ([29]; the present study). In contrast, seizures induced by local anesthetics such as cocaine or lidocaine are manifested primarily as forelimb clonus, indicative of a weaker, sub-maximal convulsion, and 1–3 of these seizures do facilitate subsequent limbic kindling [13,29].

These observations, although far from conclusive, suggest that the type of seizure activity produced by a convulsant agent is a major determinant of whether or not it will produce a kindling effect. This is not to deny the importance of other factors. For example, the anatomical distribution of seizure activity produced by a drug is likely to influence its ability to facilitate electrical kindling at a specific site. In addition, Wasterlain *et al.* [32] found that some types of chemically-induced focal seizures apparently do not produce a kindling effect. Nonetheless, more careful attention to the type of seizure activity produced by convulsant agents should lead to a clearer understanding of the role which seizure activity plays in kindling.

Kindling Effects on PTZ-Induced Convulsions

Cain [6] found that kindling of the AMYG and other sites significantly increased the duration and severity of PTZ-induced convulsions. Rats in the present study were to be tested for their response to PTZ after kindling but the test was discontinued because of a high mortality rate (33%). The five animals that survived Convulsion 4 and had been previously treated with PTZ showed a significant increase in convulsion duration at Convulsion 4. This finding indicates, in agreement with Cain [6], that animals are more sensitive to the convulsant effects of PTZ after kindling.

Conclusions

The results of the present study indicate that three PTZ-induced generalized convulsions produce long-term changes in brain function which enhance the expression of subsequent motor seizures without facilitating the development of limbic kindling. Other research indicates that a subconvulsive dose of PTZ has temporary effects of a similar nature. The sensitization to PTZ and facilitation of kindling that is produced by repeated IP administration of high subconvulsive doses of PTZ is a distinctly different phenomenon which resembles electrical kindling and may depend upon the occurrence of focal seizure activity. Further investigation of the differential effects of various types of seizure activity, ranging from isolated focal spikes to sustained generalized seizures, on subsequent kindling and motor seizure expression may yield substantial insight into the nature of the kindling process.

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