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Picrotoxin-Induced Behavioral Tolerance and Altered Susceptibility to Seizures: Effects of Naloxone

JOCELYN THOMAS,[1] WILLIAM L. NORES AND RHONDA PARISER

Department of Psychology, University of New Orleans, Lakefront, New Orleans, LA 70148

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THOMAS, J., W. L. NORES AND R. PARISER. *Picrotoxin-induced behavioral tolerance and altered susceptibility to seizures: Effects of naloxone.* PHARMACOL BIOCHEM BEHAV 45(3) 619–622, 1993.—The role of opiate mechanisms in the development of tolerance and altered susceptibility to seizures after repeated injections of picrotoxin was investigated. Independent groups of rats were pretreated with naloxone (0.3, 1.0, 3.0, and 10.0 mg/kg) or the saline vehicle and then tested for seizures induced by picrotoxin. The procedure was performed on 3 days at 1-week intervals, for a total of 3 testing days. Latencies to different types of seizures, the duration of postseizure immobility, and the number of focal seizure episodes were scored. In the vehicle-treated group, repeated picrotoxin injections led to an increased susceptibility to myoclonic and focal seizures and to decreased duration of postseizure immobility. Naloxone pretreatment significantly decreased the duration of the postseizure akinetic periods in the 1.0- and 10.0-mg/kg groups across all days, suggesting that endogenous opiates are involved in postseizure immobility and that there are interactions between opiate and picrotoxin mechanisms in some seizure-related behaviors. Naloxone did not alter the development of tolerance or sensitivity, indicating that naloxone-insensitive opiate mechanisms or nonopiate mechanisms may be involved in these processes.

Naloxone Seizures Picrotoxin Tolerance Kindling

PICROTOXIN is a GABA antagonist that induces a pattern of seizures that can be reliably categorized and scored behaviorally (19). Pilot investigations in our laboratory revealed that one injection of a low dose of picrotoxin produces an increase in seizure susceptibility to that same dose that is evident even when subsequent seizure testing occurs several weeks after the first injection (unpublished observations). The changes in seizure susceptibility were evident in the shorter latencies to seizures, in the increased number of focal seizure episodes, and in the occurrence of more severe seizure types at low doses of the convulsant drug. Prior treatment with picrotoxin also resulted in a decrease in the duration of the akinetic period that predictably follows picrotoxin-induced focal seizures and is characterized by immobility, loss of orienting response to environmental stimuli, and profuse salivation. These observations suggest that two different changes in responsiveness to picrotoxin occur with repeated injections. The shorter seizure latencies and increased number of focal and other seizure episodes suggest that rats become more sensitive to picrotoxin with repeated injections. However, decreases in the duration of the akinetic period that follows focal seizures suggest that animals develop tolerance to this picrotoxin-induced behavioral effect with repeated injections.

The mechanisms that underlie the changes in responsiveness to picrotoxin are not known. However, the development of susceptibility to seizures and postseizure immobility have been studied by other investigators in the electrical and pharmacological kindling models of seizures. In these models, repeated electrical stimulation at selected sites in the brain or repeated injection of convulsant drugs produce increases in susceptibility to seizures (8,12,14,16,17). The behavioral characteristics associated with kindled seizures and postseizure behavioral depression in kindled rats as described by others (1,7,10) are similar to the behavioral signs of focal seizures and postseizure akinesis induced by picrotoxin. Thus, it is reasonable to assume that seizures that are behaviorally similar are mediated by similar brain processes or mechanisms.

Opioids have been found to play a role in the kindling of seizures and in postseizure immobility. Repeated injections of opiate peptides at specific sites within the brain have been reported to lead to the display of progressive signs of behavioral seizures followed by periods of immobility (1,3). In addition, prior kindling with opiate peptides has been found to facilitate electrical kindling (1). While tolerance has been reported to develop to the epileptogenic effects of opiate agents injected intraventricularly, tolerance does not develop to the effects of opiate peptides injected intracerebrally (1–3). Naloxone has been reported to reduce the duration of postseizure

[1] To whom requests for reprints should be addressed.

immobility (7,10,18), but has weak to no effects on the kindling of seizures (5,6).

The possibility of an opiate–picrotoxin interaction is suggested by findings showing that morphine potentiates the effects of picrotoxin in some seizure-related behaviors. For example, relative to control-treated rats morphine-pretreated rats have been found to have significantly shorter latencies to behavioral seizures (9,15), significantly more seizure episodes (9,15), and significantly longer immobile periods after focal seizures (15). Thus, endogenous opiates, released during the occurrence of picrotoxin-induced seizures, may play a role in the development of altered susceptibility to seizures and in the development of tolerance to postseizure immobility effects. One objective of the present investigation was to study the effects of repeated picrotoxin injections on specific types of seizures and seizure-related behaviors. A second objective was to investigate the effect of naloxone, the prototypical opiate antagonist, on observed changes in responsiveness to picrotoxin with repeated injections.

METHOD

Subjects

Male Long-Evans hooded rats (260–375 g) were studied. Animals were maintained on a 12 L : 12 D cycle (light on at 0800 h) with food and water ad lib. Animals were handled for 2 min per day for 2 weeks prior to the start of the experiment.

Drugs

Naloxone (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.9% saline in concentrations of 0.03, 1.0, 3.0, or 10.0 mg/ml and injected in concentrations of 1 ml/kg. Picrotoxin was freshly mixed immediately prior to each test session and used within 3 h after mixing.

Procedure

Prior to seizure testing, independent groups of rats (n = 5–6) were pretreated with either naloxone (0.03, 1.0, 3.0, or 10.0 mg/kg, IP) or the saline vehicle. Thirty minutes after the pretreatment, animals were injected (SC) with picrotoxin (3.0 mg/kg), then placed in a transparent observation box and observed for 45 min for behavioral signs of seizures (described below). Pretreatment regimens and seizure testing were repeated twice at 1-week intervals for a total of 3 testing days for all groups. Each independent group of animals was given the same pretreatment dose on testing days 2 and 3 that they received on day 1.

Scoring of Seizures

Five categories of seizure-related behavior that have been identified in association with picrotoxin (19) were scored: a) generalized myoclonic seizures, characterized by quick, whole-body twitches and jerks; b) focal seizures, characterized at the onset by an arching of the head and trunk forward, followed by asymmetrical tonic or clonic activity in individual limbs or in the trunk, and the maintenance of postural control in the unaffected body regions; half or full rearing behavior in the presence of forelimb clonus; c) akinetic period, characterized by "frozen," sometimes abnormal postures, a loss of righting ability, staring, and loss of orienting response; d) generalized seizures with tonic and clonic components (GTC) seizures, characterized by a loss of normal postural control, bilaterally asymmetrical, intermittent contractions or extensions of limbs and trunk, tremor, and uncoordinated movement about the test chamber; and e) generalized clonic seizures, characterized by loss of postural control, rapid, prolonged clonic activity of fore- and hindlimbs and the torso. All behavior was monitored at the time of testing, as well as videotaped for later review and scoring.

Behavioral parameters scored included the latency to the first occurrence of myoclonic, focal, and GTC seizures; the latency to the onset of akinesis following the first focal seizure; the incidence of GC seizures; the duration of the period of akinesis that followed the first focal seizure; and total number of focal seizure episodes.

Data Analyses

All data were analyzed by a two-factor mixed analysis of variance (ANOVA) (naloxone dose × treatment day) with repeated measures on treatment day. Tukey and Dunnett posthoc tests were used for comparing means associated with significant treatment effects.

RESULTS

Figure 1 shows the mean number of focal seizures in each of the naloxone pretreatment groups on each day of seizure testing. As shown, there was a significant increase in the mean number of focal seizures over the 3 test days, $F(2, 48) = 12.74$, $p < 0.0001$, indicating that animals developed an increased sensitivity to the effects of picrotoxin on focal seizures. Posthoc comparisons of the mean number of focal seizures over the 3 test days indicated that the mean number of focal seizure episodes across all groups increased significantly ($p < 0.05$) from the first session (day 1) to the third (day 3). There was not a significant change in the mean number of focal seizure episodes between days 1 and 2 nor between days 2 and 3. Naloxone pretreatment did not have a significant effect on the development of sensitivity to focal seizures, and the naloxone dose × day of testing interaction was not significant.

Similarly, increased sensitivity to picrotoxin was also indicated by the decrease in latencies to myoclonic seizures over the experimental period. Figure 2 shows that there was a

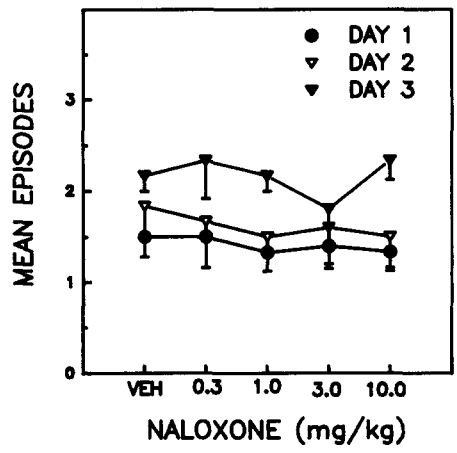

FIG. 1. Mean number of focal seizure episodes for rats pretreated with naloxone (0.03, 1.0, 3.0, or 10.0 mg/kg) or the vehicle and tested with picrotoxin (3 mg/kg) three times at 1-week intervals. Data shown represent mean ± SEM.

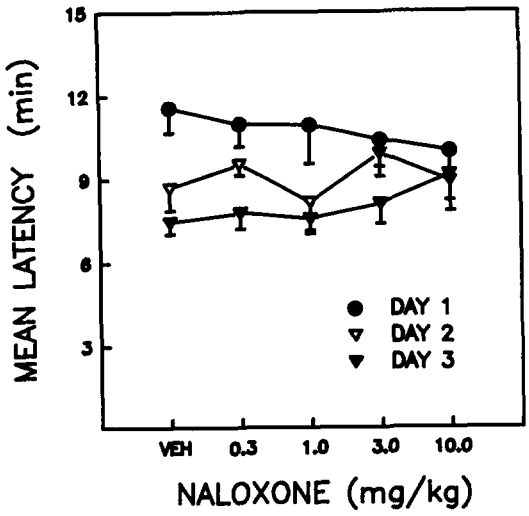

FIG. 2. Mean latencies to myoclonic seizures for rats pretreated with naloxone (0.03, 1.0, 3.0, or 10.0 mg/kg) or the vehicle and tested with picrotoxin (3 mg/kg) three times at 1-week intervals. Data shown represent mean ± SEM.

significant decrease in the latencies to these seizures for all groups over the days of testing, $F(2, 48) = 16.46$, $p < 0.0001$. Posthoc comparisons revealed that the mean latencies to myoclonic seizures on days 2 and 3 were significantly less than the mean latency on day 1 ($p < 0.05$). The latencies to focal and GTC seizures and the latency to the postfocal seizure akinesis, however, were not significantly affected by repeated picrotoxin injections over the three test sessions in any of the groups. Naloxone pretreatment did not significantly affect the latency to seizures at any of the dose levels.

The mean duration for the period of akinesis following the first focal seizure is shown in Figure 3 for all doses of naloxone on each day of testing. There was a significant decrease in the mean duration of postseizure akinesis over the three test sessions in all groups, $F(2, 48) = 11.93$, $p < 0.0001$, suggesting that tolerance develops to the effects of picrotoxin on this measure. Posthoc analyses indicated that the development of tolerance was rapid, with the mean duration of postseizure akinesis for all groups decreasing significantly between day 1 and day 2 ($p < 0.05$) and between day 1 and day 3 ($p < 0.05$) but not changing significantly between test days 2 and 3. The ANOVA also showed that there was a significant difference in the duration of postseizure akinesis among the groups pretreated with different doses of naloxone, $F(4, 24) = 2.98$, $p < 0.04$. Posthoc comparison of the mean durations of the akinetic period in the naloxone pretreatment groups with the mean duration of the control treatment group revealed that the postseizure akinetic period over all three test sessions seizures was significantly shorter in rats pretreated with the 1.0- and 10-mg/kg doses of naloxone than the postseizure akinesis across all three sessions in the control group ($p < 0.05$). As shown in Fig. 3, this was especially true on day 1, with smaller decreases over subsequent sessions. However, the naloxone pretreatment dose × day of testing interaction was not significant.

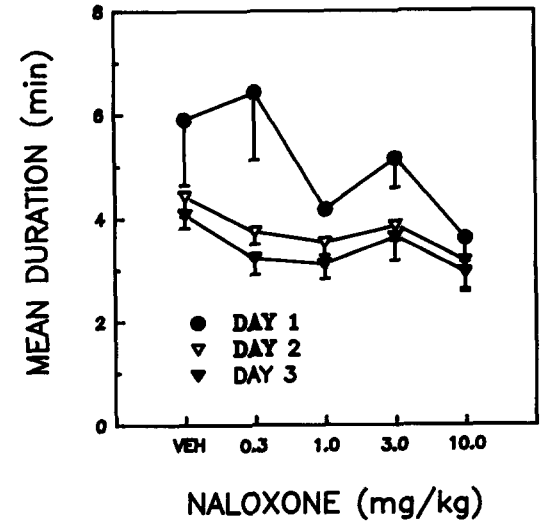

FIG. 3. Mean duration of postfocal seizure akinesis for rats pretreated with naloxone (0.03, 1.0, 3.0, or 10.0 mg/kg) or the vehicle and tested with picrotoxin (3 mg/kg) three times at 1-week intervals. Data shown represent mean ± SEM.

DISCUSSION

The results of this study suggest that several mechanisms and/or processes are affected by repeated injections of picrotoxin at the dose tested and within the time frame studied. First, repeated picrotoxin injections led to the development of tolerance to the postseizure akinetic effects of the drug but to the development of a heightened susceptibility to two specific categories of seizures—myoclonic seizures and focal seizures. Second, the rate at which tolerance to the akinetic effects and heightened susceptibility to seizures developed was different. Tolerance to postseizure immobility developed between the first and second treatments, but changes in susceptibility to focal seizures developed more gradually over the 3 treatment days. These differences suggest that the two phenomena are mediated by different mechanisms. Third, differences in the rates at which altered susceptibility to myoclonic and focal seizures developed also suggest that the mechanisms involved in these changes are not the same for both seizure categories. Increased susceptibility to myoclonic seizures was observed on the second and third test days, but a heightened sensitivity to focal seizures did not occur until the third treatment day. Further, while selected doses of naloxone significantly decreased the duration of the postseizure akinetic period, naloxone did not have any effects on the development of tolerance nor on the development of increased susceptibility to either focal or myoclonic seizures.

On the basis of similarities in the behaviors affected by kindling with picrotoxin and characteristics of opiate-kindled seizures [(1-3); unpublished observations], and on the basis of findings showing an interaction of morphine and picrotoxin (9,15), picrotoxin was hypothesized to interact with endogenous opiate systems. The effects of the 1.0- and 10.0-mg/kg doses of naloxone on the duration of postseizure immobility tends to support the idea that opiates are involved in picrotoxin-induced immobility. This finding is in agreement with the results of investigations showing that naloxone could decrease the duration of postseizure immobility in the electroshock (13) and kindling (7,10) seizure models. However, the ineffectiveness of naloxone against the development of tolerance to picrotoxin-induced immobility, despite evidence of an interaction

with the mechanisms involved in this behavior, suggests that the development of tolerance is mediated by a naloxone-insensitive opiate mechanism or a dissociable, nonopiate mechanism.

The lack of an effect for naloxone on the development of heightened seizure susceptibility is consistent with findings from investigations using the electrical and opiate kindling models (1,2,6). However, an observation that argues against the interpretation that naloxone is completely ineffective in the modulation of seizure activity is our observation that naloxone pretreatment resulted in less severe myoclonic jerks and briefer, more attenuated, focal seizures on the first and subsequent test days. Other investigators also reported weak effects for naloxone on seizure severity in kindling models (5,18). One explanation that has been offered to explain the lack of effect of naloxone on the kindling process is that so many neurotransmitter systems are involved in kindling-induced susceptibility to seizures (4,11) that antagonism of any one system would not necessarily block or retard the development of increased susceptibility (1). Another possible explanation is that the mechanisms underlying the development of heightened susceptibility to seizures and those associated with the actual precipitation or modulation of seizures could be dissociable, that is, related to different neurochemical effects. Thus, it would be possible for naloxone to have effects on the incidence, duration, or severity of seizures without effecting changes in the disposition to seizures with kindling. Additional studies are needed to identify the mechanisms by which opiate-picrotoxin interactions occur in focal and myoclonic seizures and the mechanisms involved in the development of picrotoxin-induced tolerance and sensitivity.

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