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Decreased Severity of Ethanol Withdrawal Behaviors in Kainic Acid-treated Rats

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MATSUMOTO, I., M. DAVIDSON, M. OTSUKI AND P. A. WILCE. *Decreased severity of ethanol withdrawal behaviors in kainic acid-treated rats.* PHARMACOL BIOCHEM BEHAV **55**(3) 371–378, 1996.—The involvement of kainate (KA)-sensitive regions in ethanol withdrawal behaviors was investigated in male Wistar rats given three intraperitoneal (IP) injections of KA (12 mg/kg) or saline each followed by recovery at 4°C for 5 h and room temperature for 3 days and a final KA or saline injection at room temperature. Some animals received MK-801 (1 mg/kg, IP) 30 min after each injection and one group received saline only. The saline/saline, saline/MK-801, and KA/MK-801 groups displayed typical ethanol withdrawal behaviors 8–12 h after ethanol withdrawal. These behaviors were attenuated in the KA/saline group. Audiogenic seizures could be induced in all treatment groups 12 h after withdrawal. There was severe neuronal degeneration in the hippocampal CA region and the piriform cortex of the KA/saline-treated animals that was reduced by MK-801 treatment. The inferior colliculus remained intact. These results suggest that the *N*-methyl-D-aspartate receptor mediates KA-induced damage in limbic structures and that these regions may play an important role in typical, but not audiogenically induced ethanol-withdrawal behaviors. **Copyright © 1996 Elsevier Science Inc.**

Ethanol withdrawal Hippocampus Piriform cortex Kainic acid Seizure *N*-methyl-D-aspartate

THE alcohol withdrawal syndrome is a collection of symptoms observed in individuals who stop drinking after continuous heavy consumption. Behavioral and physiological events constituting the alcohol withdrawal reaction may reflect the release of a latent state of increased neural excitability developed as a result of continuous exposure to alcohol. Specific behaviors following ethanol withdrawal are likely to be related to changes in the excitability of the particular neuronal elements mediating these individual behaviors. Gonzalez et al. reported that specific behavioral responses have temporally distinct patterns following ethanol withdrawal, suggesting that the systems responsible for these behaviors may respond independently (11). Frye et al. reported differences in the effects of pharmacological manipulations on handling-induced forelimb tremors and audiogenic seizure in ethanol-withdrawn rats (8). These results support the concept that seizure sensitivity during ethanol withdrawal may involve multiple, but independent neuronal mechanisms. Activity of these different circuits may be under the regulatory control of distinct subcortical regions.

In experimental animals, ethanol withdrawal-induced behaviors are categorized into two types: spontaneous behavior and audiogenic seizure. The adaptation in γ-aminobutyrate receptor-mediated inhibitory systems in the inferior colliculus (IC) could play an important role in the genesis of audiogenic seizures in ethanol-dependent rats (8–10). The brain regions and the transmitter systems responsible for the generation of spontaneous withdrawal behaviors are yet to be fully investigated. The limbic system, in particular the hippocampus, has been implicated in the acute effects of ethanol (16,18,19,32) and may be involved in seizure activity including that during withdrawal (39,41). No direct evidence supporting this concept has been reported, thus leaving the issue controversial (23).

Kainic acid (KA), a cyclic analogue of glutamate, when injected systemically in rats, evokes seizures accompanied by nerve cell damage primarily in the limbic system (38). After systemic administration, a variety of regions including the hippocampus, the amygdaloid complex, the piriform cortex, and several thalamic nuclei are damaged. In the hippocampus, pyramidal neuron loss is obvious in both the CA1 and CA3 subfields, with the CA1 region suffering most injury (29). It has been shown that both KA-induced behaviors and damage are temperature dependent (1,17). In the present study, by

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manipulating body temperature, we limited KA-induced damage to the CA region of the hippocampus and part of the piriform cortex. Using this model, we have investigated the contribution of KA-sensitive neurons in these two brain regions to ethanol withdrawal behaviors.

METHOD

All experimental procedures were approved by the Animal Experimental Ethics Committee of the University of Queensland.

Multiple Treatments With KA

Adult male Wistar rats, weighing approximately 200 g (233 ± 3 g, mean ± SEM), were obtained from the Central Animal Breeding House, University of Queensland, housed in a 12 D: 12 L cycle and given free access to food and water. Four treatment protocols were used. In each protocol animals received three intraperitoneal (IP) injections of either KA (12 mg/kg in 0.5 ml phosphate-buffered saline, pH 7.2, PBS) or vehicle with initial recovery at 4°C and one injection of either KA or vehicle followed by initial recovery at room temperature. Two groups of animals (n = 24 and 12) were injected with KA. One of these groups (n = 12) was injected with the noncompetitive NMDA receptor antagonist MK-801 (5-methyl-10,11-dihydro-5H-dibenzo[a,b]cyclo-hepten-5,10-imine hydrogen maleate, 1 mg/kg, IP in 0.5 ml PBS) 30 min after KA. Treatment with MK801 30 min after KA completely protects against seizure and neuronal damage in the hippocampus (2). The other group (n = 24) received saline. A further group (n = 12) received an equivalent volume of PBS instead of KA, and MK801. The final group (n = 9) received two PBS injections. All animals were housed four or five per cage, unrestrained, at 4°C during the initial 5 h after injection. This period included the peak period of seizure in the KA-treated animals. Recovery was subsequently at room temperature for 3 days. The procedure was repeated twice more. Three days after the third injection, all groups of animals were challenged with their treatment (i.e., KA or saline) and observed at room temperature.

Ethanol Treatment of Rats by Vapor Inhalation

After four treatments and 10 days of recovery, animals were made dependent by an ethanol inhalation paradigm as previously described (21,42). Briefly, rats were exposed to increasing concentrations of ethanol vapor over a 10-day introductory period, followed by an 11-day exposure at a concentration sufficient to maintain a blood ethanol level (BEL) over 200 mg/dl. BELs were determined daily using the enzymatic method of Lundquist (20). During the inhalation period, body weight and consumption of food and water were monitored periodically. On day 21 of ethanol treatment, all animals were withdrawn by removing ethanol from the air supply. Behaviors were monitored during the period of withdrawal, using the scoring system described below.

Scoring of Behavioral Changes During Ethanol Withdrawal

The scoring system for withdrawal behaviors was modified after Karanian et al. (15). Animals were scored as follows: SCORE 0, no reaction, normal behavior; SCORE 1–4, behavioral scoring for tail stiffening (1) tail tremor (1) body tremor (1) and whole body rigidity (1) were assessed by careful observation; SCORE 5, rats were then assessed for handling-induced seizure by spinning the rat once gently through a 180° arc before being placed back in the cage. In our hands no rat achieved a score of 5 without showing the previous four behaviors; SCORE 6, as above with a handling-induced continuous seizure; SCORE 7, spontaneous seizure (tonic–clonic, no handling involved) without death; SCORE 8, spontaneous seizure (tonic–clonic, no handling) with death. Scores were monitored 0, 4, 8, 10, 12, and 24 h after withdrawal by two investigators "blind" to the treatment of animals. In our hands, repeated handling of animals at different times had no effect on subsequent responses.

Assessment of Audiogenic Seizure Activity During Ethanol Withdrawal

In a separate experiment, groups of animals (n = 5) treated as above were assessed for susceptibility to audiogenic seizures 12 h into ethanol withdrawal. Seizure susceptibility was determined by stimulating individually confined rats to an electric bell (98 dB) for 1 min as described previously (10). Sound-susceptible rats exhibited one or more episodes of wild-running often leading to loss of upright posture and tonic–clonic convulsions. Two investigators without knowledge of the treatment assignments, evaluated the test.

Histological Assessment of Cell Damage

One week after the final KA injection or in the case of ethanol dependent rats, 1 week after withdrawal, animals under deep ether anesthesia were perfused through the left cardiac ventricle with 100 ml of PBS followed by 100 ml of 4% (w/v) paraformaldehyde in PBS. Brains were fixed in the same solution overnight before being cryoprotected in 30% (w/v) sucrose. Sagittal and coronal sections (50 μm) were cut on a cryostat. Sections were stained for Nissl substance using thionin and mounted for light microscopy. All sections were analyzed for major cell loss by gross assessment by two "blind" investigators.

[^{3}H]MK-801 Binding Assays

Animals were killed by decapitation 7 days after either the final injection or after completion of ethanol withdrawal paradigm. The hippocampal formation and the area including the piriform cortex and the amygdaloid complex were immediately dissected on ice. The binding assays were modified from the method described by Foster and Wong (7). Tissue was homogenized in 10 vol of 0.32 M sucrose. The homogenate was centrifuged at 1000 × g for 10 min. The pellet was resuspended in 0.32 M sucrose and centrifuged again at 1000 × g. Pooled supernatant was centrifuged at 30,000 × g for 20 min at 4°C. The pellet was resuspended in 20 vol of 5 mM Tris-HCl (pH 7.7) at 4°C and centrifuged at 30,000 × g for 20 min. Pellets were resuspended in ice-cold distilled water and centrifuged at 30,000 × g for 20 min three times before pellets were frozen at −80°C. On the day of the assay, pellets were thawed, resuspended in Tris buffer, and centrifuged at 30,000 × g for 20 min at 4°C. Pellets were washed with Tris buffer a total of four times before being resuspended in Tris buffer to give a protein concentration of 0.5 mg/ml. Aliquots (200 μl) of membrane suspensions were incubated for 2 h at room temperature with 1 μM glutamate and 1 μM glycine and six different concentrations of [^{3}H]MK-801 (NEN, Boston, MA; 20.3 Ci/mmol) ranging from 0.2–6 nM (total volume 0.5 ml). Nonspecific binding was defined as binding in the presence of 50 μM unlabeled MK-801. Incubation was terminated by filtration through Whatman GF/B glass fibre filters, presoaked in 0.3% (w/v) polyethylenimine in Tris buffer, followed by three washes with 5 ml Tris buffer. Radioactivity on the filter

was measured by liquid scintillation counting using 4 ml of Ready Protein + Scintillant (Beckman) at a counting efficiency of 50%. The binding parameters K_d and B_{max} were determined using the LIGAND program (28).

Statistical Analysis

One-way analysis of variance followed by comparison of groups by Student–Newman–Keuls method was used for statistical analysis of data. $p < 0.05$ was considered to indicate a significant difference. For comparison of behavioral responses after KA treatment and during ethanol withdrawal, a Fisher's exact probability test was used with $p \leq 0.05$ indicating a significant difference.

RESULTS

Behavioral and Histological Assessment

Approximately 45 min after the first injection of KA, rats exhibited intermittent masticatory movements, head nodding, praying posture and wet-dog shakes, which lasted for approximately 30 min. During the next phase between 90 and 120 min, animals exhibited repeated myoclonic twitches of the head, face and forelimbs with loss of postural control, accompanied by pronounced salivation and production of foam at the mouth. These behaviors were observed in all animals during the initial 5 h of cold exposure following KA injection. Masticatory movements and head nodding behaviors were observed in a number of the KA-treated animals (13 out of 25) 24 h after injection. Abnormal behaviors had completely disappeared by day 3 after injection. None of these animals showed tonic–clonic seizures at any time. One of the 25 animals died during the initial stage of the response.

The second injection of KA produced similar behavioral changes with decreased intensity. Some animals showed no behavioral responses (7 out of 24, $p < 0.01$ compared to the response to the first injection, Fisher's exact probability test). The number of animals (3 out of 24) showing abnormal behaviors 24 h after injection also decreased ($p < 0.01$ compared to the response to the first injection, Fisher's exact probability test). The majority of the animals (18 out of 24, $p < 0.01$ compared to the response to the second injection, Fisher's exact probability test) failed to respond to the third injection. No behavioral changes were detected in any animals 24 h after this injection. To confirm that all behavioral responses to KA had been abolished irrespective of temperature and that a consistent degree of damage in KA-sensitive brain area had been achieved, a fourth injection was followed by recovery at room temperature. No behavioral response was apparent at any time, in any animal after this injection.

In contrast to the single mortality during the cold treatment, when naive animals were given a single injection of KA (12 mg/kg) at room temperature a high mortality (15 out of 20, $p < 0.001$ compared to the response to the first injection and cold treatment, Fisher's exact probability test) from severe limbic and/or tonic–clonic seizures was observed.

Animals given MK-801 after KA exhibited wet-dog shakes 1 h after the first administration of KA. No seizure-related behaviors were observed thereafter; however, ataxia and an altered pattern of breathing was evident up to 3 h after the injection. A similar response was also observed in the saline/MK-801 group. The second, third, and fourth treatment with KA/MK-801 produced isolated wet-dog shakes and some masticatory movements. Ataxia was always initially present after each MK-801 injection.

During treatment, the KA/saline group had a reduced gain in body weight compared to the other groups up to 10 days after the initial treatment (data not shown). There was no significant difference between groups thereafter and during the ethanol vapor regime (data not shown).

The brains from the animals treated with KA/saline at 4°C had severe neuronal degeneration and cell loss in part of the piriform cortex and the CA1 subfield of the hippocampus but no damage to the inferior colliculus (Fig. 1). Treatment with MK-801 markedly reduced damage in both regions (Fig. 1). In contrast to the results in the cold-treated animals, treatment with a single injection of KA at room temperature (survival rate; 5 out of 20) resulted in more expansive brain damage. Total loss of neurons was observed in the CA1 and CA3 subfields of the hippocampus and throughout the amygdaloid complex, entorhinal cortex, and the piriform cortex (Fig. 2). Neuronal damage was not further increased in any of the cold-treated groups after experiencing ethanol withdrawal (data not shown).

Ethanol Withdrawal

No significant difference in food and water consumption and in body weight gain was observed between the groups during ethanol treatment (data not shown). There was no difference between the groups in BELs measured immediately before ethanol withdrawal ($p = 0.45$, ANOVA followed by post hoc analysis KA/saline; 353 ± 10 mg/dl; saline/saline 356 ± 26 mg/dl; saline/MK-801 425 ± 52 mg/dl; KA/MK-801 411 ± 51 mg/dl, mean ± SEM, $n = 6$–11).

In agreement with our previous results (21,42), unlesioned ethanol-treated animals displayed typical withdrawal behaviors. These behaviors became most obvious 8–24 h after withdrawal (Fig. 3, Table 1). Both the saline/MK-801 and KA/MK-801 groups showed marked behavioral responses undistinguishable from the saline/saline group (Fig. 3, Table 1). Of these behaviors tail stiffening, body, and tail tremors were the most significant. In contrast, withdrawal behaviors were significantly attenuated by multiple KA treatments (Fig. 3, Table 1). This group of animals had significantly reduced tail tremors, tail stiffening, and body tremors compared to all other groups. Interestingly, whole body rigidity was not significantly effected by the multiple KA treatment. Handling-induced and spontaneous seizures were not observed in the KA/saline-treated group but were observed in approximately 10–30% of animals from the other groups (Table 1). A high proportion of the KA/saline-treated animals failed to show any withdrawal behaviors. In contrast, all animals in the other groups showed obvious withdrawal behaviors at 12 and 24 h after withdrawal (Table 2). At earlier time points the KA/saline group had a significantly greater proportion of nonresponders than either the saline/saline or saline/MK-801 groups (Table 2).

In a separate experiment, groups of five animals were treated with the protocols previously described and were made dependent on ethanol. These animals were challenged with an audiogenic stimulus 12 h after withdrawal to assess their susceptibility to audiogenic seizure. All animals from all groups showed a typical seizure activity (wild running leading to tonic–clonic seizure) immediately after stimulation. In the first withdrawal experiment multiple testing of animals was assessed whereas the audiogenic experiment was performed with one test at 12 h. Previous experiments have indicated that in ethanol withdrawal experiments, repeated testing does not effect subsequent responses.

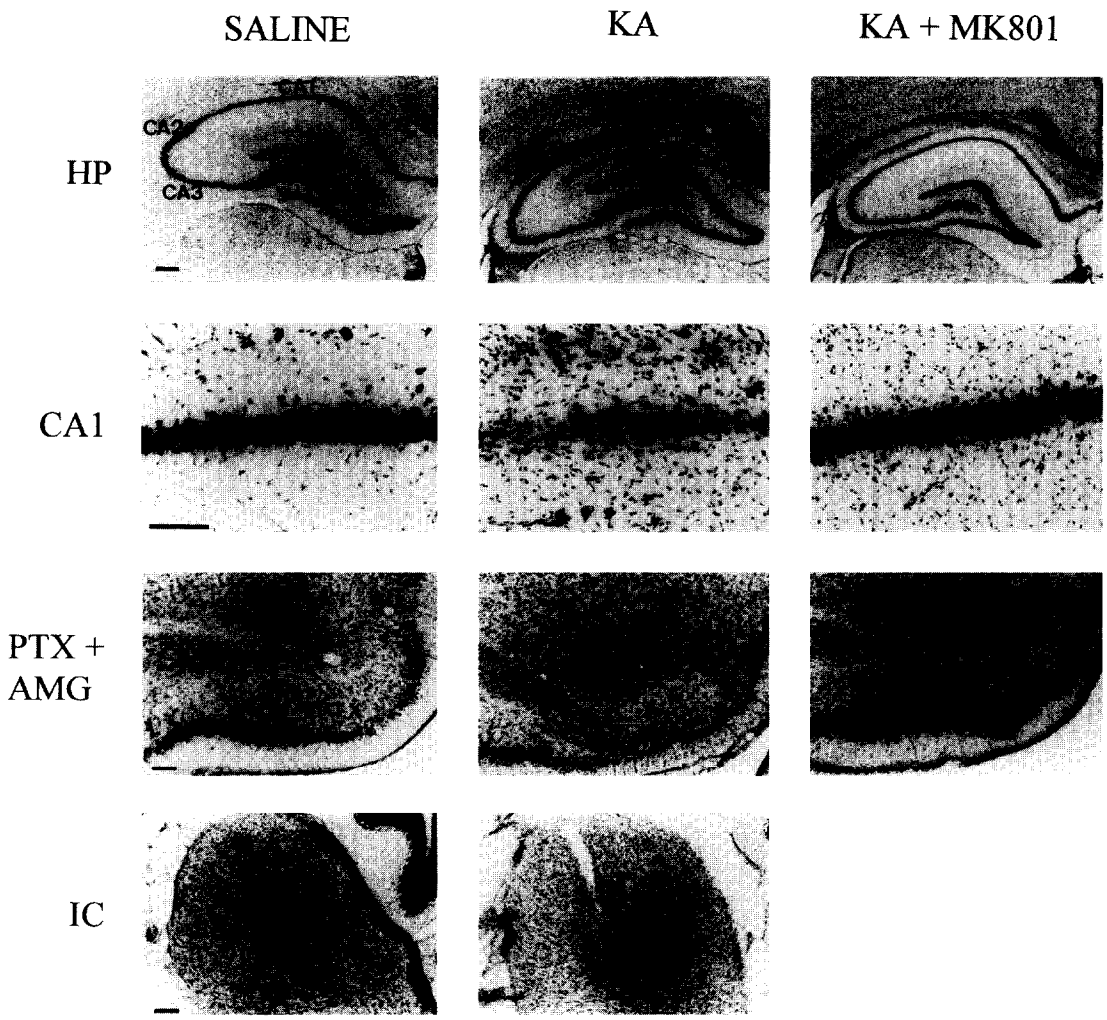

FIG. 1. Damage induced by multiple kainic acid injections followed by recovery at 4°C: the effect of MK-801. Animals received four injections of KA and, in some cases, an injection of MK-801 30 min after. Sections have been stained with thionin. HP: hippocampus; PTX+AMG: piriform cortex and amygdaloid complex; IC: inferior colliculus. Bar = 200 μm.

[^{3}H]MK-801 Binding

Tissues from the area encompassing the piriform cortex and the hippocampus were isolated from all groups to determine [^{3}H]MK-801 binding parameters as an indication of NMDA receptor density and affinity. The data from the area including the piriform cortex (Table 3) indicates a significantly lower density of binding sites in the KA/saline group. This treatment also resulted in a similar reduction in binding sites in the hippocampus (Table 4). The affinity (K_d) of the [^{3}H]MK-801 binding sites in the piriform cortex was significantly decreased in the KA/saline group (Table 3) but significantly increased in the hippocampus of the KA/MK-801–treated group (Table 4).

DISCUSSION

Specific areas of the brain have been shown to have different sensitivities to ethanol (36). At present, little evidence directly links specific brain regions to the development of the neural hyperexcitability responsible for ethanol withdrawal behaviors. We have addressed this question using animals with chemical ablation of the CA region of the hippocampus and the piriform cortex. Animals treated in this way displayed significantly fewer ethanol withdrawal-induced behaviors but audiogenically stimulated behaviors were not affected. Behaviors that were significantly affected were tail and body tremors and tail stiffening. Whole body rigidity was not affected by KA treatment.

Several studies have reported that hypothermia is effective in reducing behaviors, EEG pattern, brain damage, and mortality after KA treatment (1,17). We have confirmed this observation in the present study and have also shown that repeated injections of KA leading to chemical ablation of specific brain areas also prevents KA-induced behaviors. Neuronal cell loss and/or degeneration was restricted to the CA region, and part of the piriform cortex and mortality was markedly reduced by cold treatment. In the cold, the body temperature of saline-injected rats does not change, whereas animals receiving KA are mildly hypothermic (1). A lower brain temperature markedly inhibits the release of glutamate and other neurotransmit-

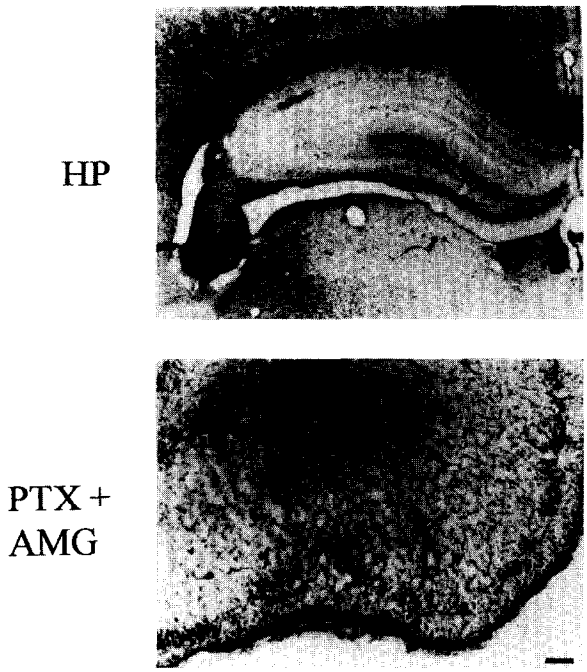

FIG. 2. Damage induced by single kainic acid (KA) injection followed by recovery at room temperature. HP: hippocampus; PTX+ AMG: piriform cortex and amygdaloid complex. Bar = 200 μm.

ters, delays the decrease in neuronal ATP levels, and reduces calcium accumulation (4). A reduction in calcium accumulation has been suggested as a mechanism by which hypothermia diminishes neuronal injury (25). A similar mechanism may account for the neuroprotective effect of cold treatment in the current study.

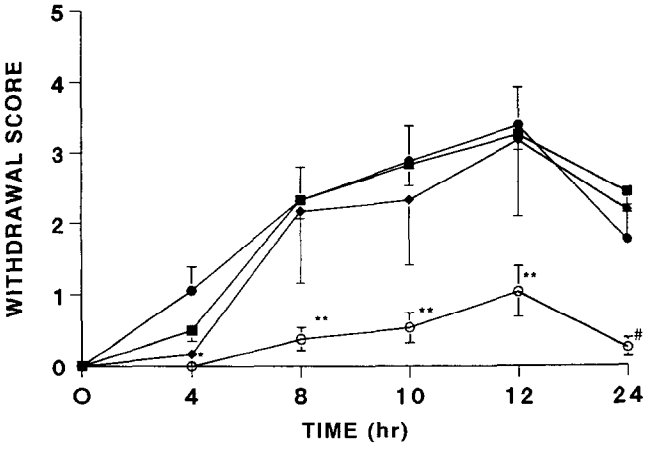

FIG. 3. Behavioral scoring during ethanol withdrawal in kainic acid (KA)- and saline-treated rats with and without treatment with MK-801. Ethanol withdrawal behaviors were monitored at the times indicated following the scoring system described in the Method section. Data presented as mean ± SEM, n = 6–12. (○) kainic acid/saline; (●) saline/saline; (■) saline/MK-801; (◆) kainic acid/MK-801. *p < 0.05 vs. saline/saline group; **p < 0.05 vs. saline/saline and saline/MK-801 groups, #p < 0.05 vs. saline/saline, saline/MK-801, and KA/MK-801 groups (Student–Newman–Keuls method).

TABLE 1

PERCENTAGE OF RATS SHOWING SPECIFIC WITHDRAWAL BEHAVIORS AT VARIOUS TIMES AFTER CESSATION OF ETHANOL TREATMENT

	% of Rats Showing Withdrawal Behavior				
	Hours				
Group and Behavior	4	8	10	12	24
Tail Tremor					
Kainic acid/saline	0	8	17	33	10
Saline/saline	44*	78*	88*	100*	88*
Saline/MK-801	0	42	42	58	42
Kainic acid/MK-801	0	17	33	33	50
Tail Stiffening					
Kainic acid/saline	0	25	25	33	20
Saline/saline	67*	100*	100*	100*	88*
Saline/MK-801	50*	100*	100*	100*	100*
Kainic acid/MK-801	17	83*	83*	83	100*
Body Tremor					
Kainic acid/saline	0	0	0	0	0
Saline/saline	0	33	38*	62*	25
Saline/MK-801	0	83*	92*	100*	92*
Kainic acid/MK-801	0	50*	50*	66*	66*
Whole Body Rigidity					
Kainic acid/saline	0	17	33	50	20
Saline/saline	33	33	63	50	13
Saline/MK-801	0	8	25	75	8
Kainic acid/MK-801	0	17	33	50	0
Seizures					
Kainic acid/saline	0	0	0	0	0
Saline/saline	0	11	13	25	0
Saline/MK-801	0	0	0	8	0
Kainic acid/MK-801	0	17	17	33	0

*p < 0.05 vs. kainic acid/saline-treated rats (Fisher's exact probability test). n = 10–12 kainic acid/saline; n = 8–9 saline/saline; n = 12 saline/MK801; n = 6 kainic acid/MK801.

KA receptors in CA1 region of the hippocampus are sparse. However, the density of NMDA and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in the CA1 is high (26,43). KA can influence the CA1 region indirectly by stimulating the CA3 pyramidal neurons, which in turn, excite the CA1 pyramidal neurons through the glutamatergic pathway of the Schaffer collaterals and comissurals (31). A pronounced release of glutamate leads to enhanced stimulation of NMDA, AMPA, and KA receptors within the CA1 region. The voltage-dependent nature of the NMDA receptor-gated channel means that the initial depolarization mediated by AMPA and KA receptors removes the Mg^{2+} block and allows ion fluxes through the channel (22,30) resulting in cell damage. In agreement with the previous report of Berg et al. (2), we have shown that KA-induced damage to the CA1 region is prevented by MK-801, which acts as an NMDA receptor open channel blocker. Further, we have shown that KA treatment results in loss of [^{3}H]MK-801 binding sites in this region. This would indicate an effect of KA on cells displaying NMDA receptors and is likely to reflect loss of these cells from the targeted tissue. The change in K_d in the KA/MK-801 group in this area suggests a complex interrelationship at play between NMDA agonists and antagonists, especially after multiple injections and may reflect selective changes in receptor subtypes under these conditions.

TABLE 2

NUMBER OF RATS IN EACH TREATMENT GROUP SHOWING NO WITHDRAWAL BEHAVIORS AT VARIOUS TIMES AFTER CESSATION OF ETHANOL TREATMENT

	Rats Showing No Withdrawal Behaviors				
	Hours				
Group	4	8	10	12	24
Kainic acid/saline	12/12	8/12	7/12	6/12	7/10
Saline/saline	2/9*	0/9*	0/8*	0/8*	0/8*
Saline/MK-801	6/12*	0/12*	0/12*	0/12*	0/12*
Kainic acid/MK-801	5/6	1/6	1/6	0/6*	0/6*

*$p \leq 0.05$ vs. kainic acid/saline treated rats (Fisher's exact probability test).

The NMDA receptors in the hippocampus are particularly sensitive to the inhibitory action of ethanol (16,18,19,32). There is accumulating evidence that, in response to this inhibition, NMDA receptors may become upregulated during chronic exposure to ethanol (3). Several recent studies have suggested that ethanol withdrawal behaviors may result from this adaptation (12–14). Furthermore, Valverius et al. demonstrated a high number of NMDA receptors in the hippocampus of mice selectively bred for susceptibility to ethanol withdrawal seizures (40). Thus, adaptation of the NMDA receptor/ionophore complex in the hippocampus may play an important role in mediating the behaviors associated with abrupt ethanol withdrawal in dependent animals. These previous observations taken together with the data presented here, strongly suggest a probable important role for the CA region of the hippocampus in at least some of ethanol withdrawal behaviors. This role may be by virtue of changes in the NMDA receptors within the region or alternatively, because the CA1 region is the exit of amplified excitation from the hippocampus, it is possible that it functions as one part of a larger neuronal network recruited to produce the withdrawal response.

The piriform cortex has been described as a crucial epileptogenic site (33). Microinjection of various convulsants, including NMDA and KA, into this region induces seizures and epileptiform activity in cortical and subcortical sites (24,35,37). NMDA receptor antagonists are able to prevent these convulsant-induced seizures when locally applied (24,34,35). Therefore, excitation of NMDA receptors may play a crucial role in the genesis of epileptogenic activity in the piriform cortex. The action of KA as a convulsant and excitotoxin in this region, may indirectly depend upon activation of NMDA receptors. It has been reported that KA exerts an action presynaptically, releasing excitatory amino acids, which result in NMDA receptor-mediated excitotoxicity (5,6). In this study, MK-801 was effective in preventing KA-induced damage in the piriform cortex while KA treatment resulted in a decreased density of MK-801 binding sites. There was a significant increase in affinity for [^{3}H]MK-801 in this area. This could reflect the loss of cells particularly sensitive to the excitotoxic actions of the administered KA and which display subtypes of the NMDA receptor. Receptors on the residual cells may have subtly different characteristics.

Although the ethanol sensitivity of the NMDA receptors in this region has not been studied, mapping studies using c-*fos* expression clearly indicates increased neuronal activity in the piriform cortex as well as the hippocampus during ethanol withdrawal (21,27). This expression was prevented by pretreatment with MK-801 (42). Therefore, the hypothesis that neuronal cells in the piriform cortex that are sensitive to NMDA play a role in the generation of ethanol withdrawal response has some support.

In the generation of audiogenic seizures in ethanol-dependent rats, the IC has been suggested to play a fundamental role. This is due to its key position in the auditory pathway. It consists of a central mass of cells (central nucleus) surrounded by a cortex (dorsal and external). Work by other groups [reviewed by Speck (38)] and ourselves failed to show KA-induced damage in either IC cortex or IC nucleus. Either bilateral injections of $GABA_A$-agonists into the IC or bilateral electrolytic lesions of the IC suppressed sound-induced sei-

TABLE 3

SCATCHARD ANALYSIS OF [^{3}H]MK-801 BINDING IN THE AREA ENCOMPASSING THE PIRIFORM CORTEX

Group	K_d (nM)	B_{max} (pmol/mg protein)
Saline/saline	1.05 ± 0.065	1.7 ± 0.12
Kainic acid/saline	0.86 ± 0.08*	1.13 ± 0.1†
Saline/MK-801	1.34 ± 0.06	1.89 ± 0.1
Kainic acid/MK-801	1.03 ± 0.13	1.8 ± 0.1

*$p < 0.05$ vs. saline/MK-801 group (ANOVA and Student–Newman–Keuls test).

†$p < 0.05$ vs. saline/saline, saline/MK-801, and kainic acid/MK-801 groups (ANOVA and Student–Newman–Keuls test). Data are mean ± SEM, $n = 4$ in each group.

TABLE 4

SCATCHARD ANALYSIS OF [^{3}H]MK-801 BINDING IN RAT HIPPOCAMPUS

Group (n)	K_d (nM)	B_{max} (pmol/mg protein)
Saline/saline (5)	0.94 ± 0.08	1.76 ± 0.08
Kainic acid/saline (4)	0.66 ± 0.05	1.17 ± 0.02†
Saline/MK-801 (13)	0.84 ± 0.05	1.75 ± 0.05
Kainic acid/MK-801 (8)	1.27 ± 0.12*	1.53 ± 0.1

*$p < 0.05$ vs. saline/saline, kainic acid/saline and saline/MK-801 groups (ANOVA and Student–Newman–Keuls test).

†$p < 0.05$ vs. saline/saline, saline/MK-801 and kainic acid/MK-801 groups (ANOVA and Student–Newman–Keuls test). Data are mean SEM.

zures of the ethanol-withdrawing animals. This has lead to a hypothesis that adaptive reductions in GABAergic inhibition in the IC could be responsible for an increased audiogenic seizure susceptibility during ethanol withdrawal (8–10). In contrast to the typical withdrawal behaviors, the occurrence and seizure pattern elicited by audiogenic stimulus was not altered in any of the KA-treated groups. No visible histological damage was observed in the inferior colliculus in treated animals. These data confirm that KA treatment does not abolish all aspects of ethanol dependence. They also emphasise that withdrawal behaviors and audiogenically induced seizures probably do not have the involvement of the CA region and the piriform cortex in common. This further supports the suggestions that these behaviors involve various independent neuroanatomical and/or neurotransmitter systems in the chronic adaptive response to ethanol.

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REFERENCES

1. Balchen, T.; Berg, M.; Diemer N. H. A paradox after systemic kainate injection in rats: Lesser damage of hippocampal CA1 neurons after higher doses. Neurosci. Lett. 163:151–154; 1993.
2. Berg, M.; Bruhn, T.; Johansen, F. F.; Diemer, N. H. Kainic acid-induced seizures and brain damage in the rat: Different effects of NMDA- and AMPA receptor antagonists. Pharmacol. Toxicol. 73:262–268; 1993.
3. Buck, K. J.; Harris, R. A. Neuroadaptive responses to chronic ethanol. Alcohol. Clin. Exp. Res. 15:460–470; 1991.
4. Busto, R.; Globus, M. Y. T.; Dietrich, W. D.; Martinez, E.; Valdes, I.; Ginsberg, M. D. Effect of mild hypothermia on ischemia-induced release of neurotransmitters of free fatty acids in rat brain. Stroke 20:904–910; 1989.
5. Collins, G. G. S.; Ansou, J.; Surtes, L. Presynaptic kainate and and *N*-methyl-D-aspartate receptors regulate excitatory amino acid release in the olfactory cortex. Brain Res. 265:157–159; 1983.
6. Ferkany, J. W.; Zaczek, R.; Coyle, J. T. Kainic acid stimulates excitatory amino acid neurotransmitter release at presynaptic receptors. Nature 298:757–759; 1982.
7. Foster, A. C.; Wong, E. H. F. The novel anticonvulsant MK-801 binds to the activated state of the *N*-methyl-D-aspartate receptor in rat brain. Br. J. Pharmacol. 91:403–409, 1987
8. Frye, G. D.; McCown, T. J.; Breese, G. R. Differential sensitivity of ethanol withdrawal signs in the rat to γ-aminobutyric acid (GABA) mimetics: Blockade of audiogenic seizures but not forelimb tremors. J. Pharmacol. Exp. Ther. 226:720–725; 1983.
9. Frye, G. D.; McCown, T. J.; Breese, G. R. Characterization of susceptibility to audiogenic seizures in ethanol-dependent rats after microinjection of gamma-aminobutyric acid (GABA) agonists into the inferior colliculus, substantia nigra or medial septum. J. Pharmacol. Exp. Ther. 227:663–670; 1983.
10. Frye, G. D.; McCown, T. J.; Breese, G. R.; Peterson, S. L. GABAergic modulation of inferior colliculus excitability: Role in the ethanol withdrawal audiogenic seizures. J. Parmacol. Exp. Ther. 237:478–485; 1986.
11. Gonzalez, L. P.; Czachura, J. F.; Brewer, K. W. Spontaneous vs. elicited seizures following ethanol withdrawal: Differential time course. Alcohol 8:481–487; 1989.
12. Grant, K. A.; Valverius, P.; Hudspith, M.; Tabakoff, B. Ethanol withdrawal seizures and the NMDA receptor complex. Eur. J. Pharmacol. 176:289–296; 1990.
13. Grant, K.; Snell, L. D.; Rogawski, M. A.; Thurkauf, A.; Tabakoff, B. Comparison of the effect of the uncompetitive *N*-methyl-D-aspartate antagonist (±)-5-aminocarbonyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5, 10-imine (ADCI) with its structural analogs dizocilpine (MK-801) and carbamazepine and ethanol withdrawal seizures. J. Pharmacol. Exp. Ther. 260:1017–1022; 1992.
14. Gulya, K.; Grant, K. A.; Valverius, P.; Hoffman, P. L.; Tabakoff, B. Brain regional specificity and time course of changes in the NMDA receptor-ionophore complex during ethanol withdrawal. Brain Res. 547:129–134; 1991.
15. Karanian, J.; Yergey, J.; Lister, R.; D'Souza, N.; Linnoila, M.; Salem, N. Characterization of an automated apparatus for precise control of inhalation chamber ethanol vapour and blood ethanol concentrations. Alcohol. Clin. Exp. Res. 10:443–447; 1986.
16. Lima-Landman, M. T. R.; Albuquerque, E. X. Ethanol potentiates and blocks NMDA-activated single-channel currents in rat hippocampal pyramidal cells. FEBS Lett. 247:61–67; 1989.
17. Liu, Z; Gatt, A.; Mikati, M.; Holmes, G. L. Effect of temperature on kainic acid-induced seizures. Brain Res. 631:51–58; 1991.
18. Lovinger, D. L.; White, G.; Weight, F. F. NMDA recpetor mediated synaptic excitation selectively inhibited by ethanol in hippocampal slice from adult rat. J. Neurosci. 10:1372–1379; 1990.
19. Lovinger, D. L.; White, G.; Weight, F. F. Ethanol inhibits NMDA-activated ion current in hippocampal neurons. Science 24:1721–1724; 1990.
20. Lundquist, F. The determination of ethyl alcohol in blood and tissue. Methods Biochem. Anal. 7:215–221; 1959.
21. Matsumoto. I.; Leah, J.; Shanley, B. C.; Wilce, P. A. Immediate early gene expression in the rat brain during ethanol withdrawal. Mol. Cell. Neurosci. 4:485–491; 1993.
22. Mayer, M. I.; Westbrook, G. L.; Buthrie, P. B. Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurons. Nature 309:261–263; 1984 .
23. McCown, T. J.; Breese, G. R. Multiple withdrawals from chronic ethanol "kindles" inferior collicular seizure activity: Evidence for kindling of seizures associated with alcoholism. Alcohol. Clin. Exp. Res. 14:394–399; 1989.
24. Millan, M. H.; Patel, S.; Mello, L. M.; Meldrum, B. S. Focal injection of 2-amino-7-phosphonoheptanoic acid into prepiriform cortex protects against pilocarpine-induced limbic seizures in rats. Neurosci. Lett. 70:69–74; 1986.
25. Mitani, A.; Kadoya, I.; Kataoka, K. Temperature dependence of hypoxia-induced calcium accumulation in gerbil hippocampus slice. Brain Res. 562:159–163; 1991.
26. Monaghan, D. T.; Holets, V. R.; Toy, D. W.; Cotman, C. W. Anatomical distributions of four pharmacologically distinct ^{3}H-L-glutamate binding sites. Nature 306:176–179; 1983.
27. Morgan, P. F.; Nadi, N. S.; Karanian, J.; Linnoila, M. Mapping rat brain structures activated during ethanol withdrawal: Role of glutamate and NMDA receptors. Eur. J. Pharmacol. 225:217–223; 1992.
28. Munson, P. J.; Rodbard, D. LIGAND: A versatile computerized approach for characterisation of ligand-binding systems. Anal. Biochem. 107:220–239; 1980.
29. Nitecra, L.; Tremblay, E.; Charton, G.; Bouillot, J. P.; Berger, M. L.; Ben-Ari, Y. Maturation of kainic acid seizure-brain damage syndrome in the rat. II. Histopathological sequelae. Neuroscience 13:1073–1094; 1984.
30. Nowak, I.; Bregestorski, P.; Ascher, P.; Herbert, A.; Prochiantz, A. Magnesium gated glutamate-activated channels in mouse central neurons. Nature 307:462–465; 1984.
31. Olney, J. W.; DeGubareff, T.; Sloviter, R. S. "Epileptic" brain damage in rats induced by sustained electrical stimulation of the perforant path. II. Ultrastructural analysis of acute hippocampal pathology. Brain Res. Bull. 10:699–712; 1983.
32. Peoples, R. W.; Weight, F. F. Ethanol inhibition of *N*-methyl-

D-aspartate-activated ion current in rat hippocampal neurons is not competitive with glycine. Brain Res. 571:342–344; 1992.
33. Piredda, S.; Gale, K. A crucial epileptogenic site in the deep prepiriform cortex. Nature 317:623–625; 1985.
34. Piredda, S.; Gale, K. Role of excitatory amino acid transmission in the genesis of seizures elicited from the deep prepiriform cortex. Brain Res. 377:205–210; 1986.
35. Piredda, S.; Gale, K. Anticonvulsant action of 2-amino-7-phosphonoheptanoic acid and muscimol in the deep prepiriform cortex. Eur. J. Pharmacol. 120:115–118; 1986.
36. Proctor, W. R.; Allan, A. M.; Dunwiddie, T. V. Brain region-dependent sensitivity of $GABA_A$ receptor-mediated responses to modulation by ethanol. Alcohol. Clin. Exp. Res. 16:480–489; 1992.
37. Sarro, G. D.; Donato, E.; Paola, D,; Sarro, A. D.; Vidal M. J. L-Arginine potentiates excitatory amino acid-induced seizures elicited in the deep prepiriform cortex. Eur. J. Pharmacol. 230: 151–158; 1993.
38. Sperk, G. Kainic acid seizures in the rat. Prog. Neurobiol. 42: 1–32; 1994.
39. Turski, W. A.; Cavalheiro, E. A.; Schwarcz, M.; Czuczwar, S. J.; Kleinrik, Z.; Turski, L. Limbic seizures produced by pilocarpine in rats: Behavioural electroencephalographic and neuropathological study. Behav. Brain Res. 9:315–335; 1983.
40. Valverius, P.; Crabbe, J. C.; Hoffman, P. L.; Tabakoff, B. NMDA receptors in mice bred to be prone or resistant to ethanol withdrawal seizures. Eur. J. Pharmacol. 184:185–189; 1990.
41. Walker, D. W.; Zornetzer, S. F. Alcohol withdrawal in mice: Electroencephalographic and behavioral correlates. Electroencephalogr. Clin. Neurophysiol. 36:233–244; 1974.
42. Wilce, P. A.; Le, F.; Matsumoto, I.; Shanley, B. C. Ethanol inhibits NMDA receptor-mediated regulation of immediate early gene expression. Alcohol Alcohol. (Suppl.) 2:359–363; 1993.
43. Young, A. B.; Fagy, G. E. Excitatory amino acid receptors in the brain: Membrane binding and receptor autoradiographic approaches. Trends Pharmacol. Sci. 11:126–133; 1990.