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A comparison of three NMDA receptor antagonists in the treatment of prolonged status epilepticus

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Abstract

Three different classes of NMDA receptor antagonists were compared for their effectiveness in terminating prolonged status epilepticus (SE), induced by continuous hippocampal stimulation. Animals were treated after 150 min of SE by intraperitoneal administration of increasing doses of 3-((*R,S*)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), MK-801 (dizocilpine), ifenprodil, or saline. EEG recordings were used to determine seizure termination. The first experiment ($n = 57$ animals) determined the most effective anticonvulsant dose of each agent by determining its ability to terminate SE within the next 300 min. Five control rats treated with normal saline after 150 min of SE continued to exhibit continuous seizures for the next 300 min. All drugs were administered after 150 min of SE. CPP terminated seizures with an ED_{50} of 6.4 mg/kg; the maximal effective dose was 15 mg/kg. MK-801 has an ED_{50} of 1.4 mg/kg; the maximal effective dose was 2 mg/kg. Ifenprodil was maximally effective at 30 mg/kg. However, an ED_{50} could not be calculated. In a subsequent experiment, the NMDA antagonists were compared for their ability to terminate prolonged SE within 60 min of their administration at the most effective dose. MK-801 (2.0 mg/kg) terminated SE in 6 of 10 animals within 60 min, CPP (15 mg/kg) terminated it in 1 of 9 animals; ifenprodil (30 mg/kg) did not terminate it in any of 9 animals treated. In the 300 min following administration, CPP (6/9) and MK-801 (6/10) were equally efficacious in terminating SE but ifenprodil (2/7) was less effective ($P = 0.065$, chi-square test). The results indicate that the non-competitive NMDA receptor antagonist MK-801 was superior to the competitive antagonist CPP and the pH-sensitive site antagonist ifenprodil, in terminating prolonged experimental SE.

Keywords

Status epilepticus; NMDA receptor; Hippocampus; CPP; Ifenprodil MK-801

1. Introduction

Prolonged, self-sustaining seizures are commonly referred to as status epilepticus (SE). Current therapy of SE focuses largely on two mechanisms, the enhancement of γ -aminobutyric-acid (GABA)-mediated inhibition by benzodiazepines and barbiturates and the modulation of sodium channels by phenytoin or fosphenytoin (Macdonald and Kelly, 1995). As many as 35–50% of patients with generalized convulsive SE are refractory to the use of GABAergic drugs (Alldredge et al., 2001; Treiman et al., 1998). In animal models and human clinical trials, phenytoin is less effective in terminating SE than GABAergic drugs (Jones et al., 2002; Treiman et al., 1998). A retrospective analysis of patients in convulsive and subtle SE in a large academic hospital found that 31% of seizures are refractory to a combination of a

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benzodiazepine and a second line agent, phenytoin, fosphenytoin, or phenobarbital (Mayer et al., 2002). There are at least 126,000 to 195,000 episodes of SE each year in the US (DeLorenzo et al., 1996). If the rate of refractory SE is conservatively estimated at 30% of all episodes, then there are about 38,000–54,500 episodes of refractory SE in the US each year. Clearly there is a need to identify new drugs to treat refractory SE.

N-Methyl-d-aspartate (NMDA) antagonists, such as ketamine and MK-801 (dizocilpine), can terminate prolonged, GABA-refractory SE in experimental animals (Bertram and Lothman, 1990; Borris et al., 2000; Mazarati and Wasterlain, 1999). Other studies indicate that the NMDA receptor antagonist MK-801 prevented the development of refractoriness to diazepam in the lithium/pilocarpine model of SE (Rice and DeLorenzo, 1999). In addition, NMDA receptor antagonists offer the possibility of protecting neurons from seizure-induced neuronal damage and the development of epilepsy (Fujikawa et al., 1994; Prasad et al., 2002). In these studies, MK-801 dose–response curves were not obtained and systematic comparisons of MK-801 with other NMDA receptor antagonists were not made.

NMDA receptor function can be inhibited by multiple mechanisms including competitive block by drugs such as 3-((*R,S*)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), by open channel block at the phencyclidine (PCP) site by MK-801 or ketamine (Collingridge and Lester, 1989), and by action at the pH-sensitive site by ifenprodil (Mott et al., 1998). It is not known whether drugs acting at different sites on the NMDA receptor are equally effective in terminating prolonged, refractory SE. We compared representative compounds belonging to these three classes of NMDA receptor antagonists for their ability to terminate prolonged SE.

2. Methods

All procedures on animals were performed according to a protocol approved by the institutional Animal Care and Use Committee. Adult male Sprague–Dawley rats weighing 250–325 g were housed with food and water ad libitum. Bipolar insulated stainless steel electrodes were implanted stereotaxically in the left posterior ventral hippocampi under ketamine/xylazine anesthesia and secured to the skull with dental acrylic, as described previously (Lothman et al., 1988). SE was induced in each rat by continuous hippocampal stimulation (CHS) (Lothman et al., 1989) with 1 ms biphasic square wave electrical pulses (50 Hz, 400 mA) in 10 cps trains applied every 11 s for 90 min during which time seizures occurred. Post-stimulation seizure activity was monitored continuously for 6 h by electroencephalogram (EEG) recordings. Only those animals that continued to have continuous seizures (electrographic stage III) for 60 min after the end of stimulation were included in the study, at which time they were either treated with normal saline or a drug. A total of 60 rats were used for the dose–response study. Animals belonged to one of three experimental groups: 16 treated CPP, 20 treated with MK-801, and 19 treated with ifenprodil. For initial experiments, drug doses were chosen based on results of previous studies. Subsequently, doses were increased or decreased to obtain the full range of the drugs' effects, from no response to maximal effect. Five animals treated with saline formed the control group. In a subsequent experiment, 14 additional animals were studied to compare the most effective dose of each NMDA receptor antagonist. All drugs were dissolved in normal saline solution.

EEG activity was recorded continuously for 5 h following drug injection to determine the effect of the drug on prolonged SE. SE was considered terminated when the EEG returned to normal baseline or showed irregular spikes without recurrence of seizures in a subsequent observation period. Data for the logarithm of drug doses and the percentage of seizure-free animals were fit to an equation for a sigmoidal curve with a variable slope. The maximum and minimum responses were fixed to 100 and 0%, respectively. The ED₅₀ of the drug was determined from the equation that best fit the data.

3. Results

3.1. Characterization of dose–response relationship

Treatment was initiated 150 min after the onset of seizures and only those animals that continued to have continuous electrographic seizures (stage III) for the entire duration were included in the study. Five control rats treated with normal saline after 150 min of seizures continued to exhibit continuous seizures for the next 300 min. The first set of experiments determined the most effective anticonvulsant dose for each of the three drugs. Increasing doses of the drugs were administered and if seizures were terminated anytime within the subsequent 300 min, the animal was considered a responder.

The competitive NMDA antagonist, CPP, was administered to 16 animals following 150 min of seizures (Fig. 1A at four doses: 2.5, 5, 10 and 15 mg/kg (four animals per group)). All four animals injected with 2.5 mg/kg CPP had SE for the following 300 min. Doubling the dose to 5 mg/kg controlled SE in 1/4 (25%) treated animals, whereas the 10 mg/kg dose resulted in the control of electrographic seizures in 3/4 (75%) animals. Termination of SE was associated with suppression of background EEG with no spikes or presence of irregular spikes with a frequency less than 2 Hz. Finally, CPP (15 mg/kg) controlled SE in all four (100%) animals. The best fit of the log dose and fraction of animals seizure-free data to the equation for a sigmoidal function suggested an ED₅₀ of 6.8 mg/kg of CPP for treatment of prolonged SE. Animals treated with CPP doses of 10 mg/kg and higher were sedated without signs of respiratory depression. One animal died in the week following SE, and 10 of the 15 (67%) remaining animals became epileptic.

MK-801 was administered to subsets of 20 animals at five doses: 0.5 mg/kg ($n = 3$), 1 mg/kg ($n = 4$), 1.5 mg/kg ($n = 4$), 2 mg/kg ($n = 4$) and 2.5 mg/kg ($n = 5$) following 150 min of seizures (Fig. 1B). SE was controlled in none of the animals treated with the lowest dose of MK-801 (0.5 mg/kg), in 1/4 (25%) animals treated with the 1 mg/kg dose, and in 2/4 (50%) animals treated with the 1.5 mg/kg dose. Administration of MK-801 (2 mg/kg) resulted in the control of SE in 3/4 (75%) animals, but had no effect on one rat, in which continuous electrographic seizures were present prior to treatment and the EEG remained unchanged following drug administration. At a dose of 2.5 mg/kg however, a loss of efficacy occurred resulting in termination of SE in only 1/5 (20%) animals. The best fit of the log dose and fraction of animals seizure-free data to the equation for a sigmoidal curve suggested an ED₅₀ of 1.4 mg/kg of MK-801 for treatment of SE. The MK-801 doses of 1 mg/kg and higher caused sedation but respiratory depression did not occur in any animal. One animal died 1 week after SE, and 10 of the 19 (53%) remaining animals subsequently developed epilepsy.

Ifenprodil was administered to subsets of 18 animals at four doses: 3 mg/kg ($n = 4$), 10 mg/kg ($n = 6$), 20 mg/kg ($n = 4$), and 30 mg/kg ($n = 4$) following 150 min of seizures (Fig. 1C). The low dose of ifenprodil (3 mg/kg) did not control SE in any of the four animals tested. SE was terminated in 1/6 (17%) animals treated with 10 mg/kg. Doubling the dose to (20 mg/kg) controlled SE in 1/4 (25%) of animals. A (30 mg/kg) dose was able to control SE in only 2/4 (50%) animals. Higher doses of ifenprodil could not be administered due to limitations of drug solubility. The data could not be fit to an equation for a sigmoid function. In the week following SE, two rats died and one was euthanized; and 9 out of the 15 (60%) remaining animals developed epilepsy.

3.2. A comparison of the most effective dose of MK-801, CPP and ifenprodil

The dose–response data suggested that MK-801, CPP, and ifenprodil effectively shortened the duration of SE. The time to terminate SE was not compared in the previous experiment, because termination of seizures any time within 300 min of drug administration was considered a

response. Because a rapid termination of SE is desirable, the most effective dose of CPP (15 mg/kg), MK-801 (2 mg/kg) and ifenprodil (30 mg/kg) were compared for their ability to terminate SE within 60 min of injection.

Five animals were treated with 15 mg/kg of CPP after 150 min of SE. Seizures did not stop in any animal within 60 min of injection but were terminated in the subsequent 240 min in two out of five animals. EEG data generated in the previous experiment in which four animals with prolonged refractory SE were treated with 15 mg/kg were reanalyzed to determine the time of termination of SE. CPP terminated SE within 60 min of injection in 1/4 (25%) animals in that group. In summary, CPP terminated SE in 1/9 (11%) animals within 60 min of injection and in 6/9 (67%) animals within 300 min of injection (Fig. 2A and B).

Six animals were treated with 2 mg/kg of MK-801 after 150 min of SE. Seizures stopped in 3/6 (50%) animals within 60 min of injection. There were no additional responders to treatment in the subsequent 240 min. EEG data generated in the previous experiment in which four animals with prolonged refractory SE were treated with 2 mg/kg MK-801 were reanalyzed to determine the time of termination of SE. MK-801 terminated SE within 60 min of injection in 3/4 (75%) animals. In summary, MK-801 terminated SE in 6/10 (60%) animals within 60 min of treatment and no other animal in the subsequent 240 min (Fig. 2A and B).

Three animals were treated with 30 mg/kg of ifenprodil after 150 min of SE. Seizures did not stop in any animal within 60 min of injection. There were no responders to treatment in the subsequent 240 min. EEG data generated in the previous experiment in which four animals with prolonged refractory SE were treated with 30 mg/kg ifenprodil were reanalyzed to determine the time of termination of SE. Ifenprodil did not terminate SE within 60 min of injection in any animal. In summary, ifenprodil terminated SE in 0/7 (0%) animals within 60 min of treatment and in the subsequent 240 min 2/7 (28%) became seizure-free (Fig. 2A and B).

Comparing the efficacy of the drugs, MK-801 (2 mg/kg) was superior to CPP (15 mg/kg) and ifenprodil (30 mg/kg) in terminating SE within 60 min of drug injection ($P = 0.0094$, chi-square test). However, MK-801 and CPP were equally effective in terminating SE within 300 min of treatment ($P = 0.736$, chi-square test). There was a trend for ifenprodil to be less efficacious than MK-801 or CPP in terminating SE within 300 min of treatment ($P = 0.065$, chi-square test).

4. Discussion

The principal findings of this study are (1) MK-801 was superior to CPP and ifenprodil in rapidly terminating SE; (2) MK-801 and CPP were equally effective in shortening the duration of SE; and (3) ifenprodil was somewhat less efficacious than MK-801 and CPP in reducing the duration of SE.

Prolonged SE was used as a model of drug refractory SE in the current study. Studies in several models of SE suggest that prolonged seizures are drug refractory (Jones et al., 2002; Kapur and Macdonald, 1997; Mazarati et al., 1998; Walton and Treiman, 1988). For example, in the lithium/pilocarpine model, benzodiazepines terminate brief seizures but SE becomes refractory to benzodiazepines within 10 min of the first grade 3 (behavioral) seizure (Jones et al., 2002). In the continuous hippocampal stimulation model of SE, seizures become refractory to GABAergic drugs such as phenobarbital after 150 min of seizures (Borris et al., 2000).

It is possible that the NMDA receptors become more sensitive to glutamate during SE. NMDA receptor activation by NMDA or glutamate is subject to regulation by several second messenger systems and protein kinases. NMDA receptor currents are potentiated by calcium

phospholipid-dependent kinase (PKC) (Friedman, 1997; Lan et al., 2001) and are inhibited by calcineurin (Dingledine et al., 1999). PKC appears to directly phosphorylate NMDA receptors and enhance NMDA receptor currents in hippocampal neurons. Prolonged seizures and SE are known to increase PKC activity in the hippocampus (Beldhuis et al., 1992; Kohira et al., 1992; Osonoe et al., 1994). Thus, it is possible that NMDA receptor sensitivity to glutamate is enhanced during SE. It was recently suggested that glutamate release is increased during recurrent bursting, this may contribute to pathogenesis of refractory SE (Mangan and Kapur, 2004). CPP may have been effective in terminating sustained SE by competitively blocking the NMDA binding site, thereby significantly decreasing the activity of NMDA receptors.

MK-801 was considered superior to CPP because it terminated SE within 60 min whereas CPP took longer to be effective. It is important to terminate SE in a prompt manner because prolonged seizures can lead to neurological and systemic complications, including pulmonary congestion and edema, cardiac arrhythmias, hypotension, elevation of body temperature, hypoglycemia, acidosis and rhabdomyolysis. These systemic complications tend to worsen as the duration of SE lengthens (Lothman, 1990; Walton, 1993). There is growing evidence from experimental animals that the extent of neuronal injury due to SE is linked to the duration of the seizures (Fujikawa, 1996; Lemos and Cavalheiro, 1996). Refractoriness of SE also worsens with the passage of time (Treiman et al., 1998; Walton and Treiman, 1988; Yaffe and Lowenstein, 1993). Mortality associated with status epilepticus also correlates with the duration of the seizures (Towne et al., 1994).

MK-801 is a well-established NMDA receptor antagonist that acts by a use-dependent open channel block mechanism. MK-801 directly blocks NMDA channels at the PCP site while the channels are open during depolarization. During prolonged SE hippocampal neurons are depolarized (Kapur and Coulter, 1995) and this depolarization is likely to open NMDA receptors and allow entry of MK-801 and the subsequent blockade of the receptors. MK-801 terminated prolonged SE in a dose-dependent fashion between doses of 0.5 and 2 mg/kg but a higher dose, 2.5 mg/kg was less effective. Ketamine, another NMDA receptor antagonist at the PCP site, demonstrated a similar loss of effectiveness at 150 mg/kg compared to a 100 mg/kg dose (Borris et al., 2000). A similar narrow dose range of efficacy was reported in a model of cocaine-induced seizures in mice when they were treated with the open channel blockers, MK-801 and memantine (Brackett et al., 2000). High doses of MK-801 may be less effective because in addition to blocking NMDA receptors both MK-801 and ketamine also block sigma receptors (Bouchard et al., 1997). There is growing evidence that sigma receptors modulate release of neurotransmitters such as glutamate (Meyer et al., 2002), and acetylcholine (Lurton and Cavalheiro, 1997; Vallee et al., 1997). It is possible that the actions of MK-801 at sigma receptors contribute to its failure at high doses.

Ifenprodil, a member of a class of phenylethanolamines, is a selective NR2B-subunit containing NMDA receptor antagonist with a unique mechanism. Ifenprodil increases the sensitivity of NMDA receptors to inhibition by protons (acidic pH), an endogenous negative modulatory action (Mott et al., 1998). This increased sensitivity to proton inhibition should protect neurons from prolonged NMDA receptor activation during seizures. Some studies have hypothesized about ifenprodil's potentially potent neuroprotective properties and its significantly low side-effect profile (Mott et al., 1998); however, this was the least effective drug tested in this study. At doses as high as 30 mg/kg, SE was controlled in only a limited fraction of rats. It is unlikely that higher doses of ifenprodil could control seizures because the dose-response relationship was relatively flat between the doses of 10 and 30 mg/kg. Other studies have also reported limited efficacy of ifenprodil in terminating seizures (Brackett et al., 2000). This limited efficacy of ifenprodil was not due to the lack of NR2B-subunits in hippocampal neurons, because this subunit is expressed in the rat hippocampus (Wang et al.,

1995). However, not all NMDA receptors contain the NR2B-subunit and this may have limited ifenprodil's efficacy.

Ifenprodil was considered less effective than other drugs because very high doses had to be used to get any effect, and it took long periods of time to terminate SE in a small fraction of animals. In animal neuroprotection studies, ifenprodil is commonly used at a dose of 10 mg/kg (Dogan et al., 1997); we found that doses of 20–30 mg/kg of ifenprodil were needed to control prolonged SE in 28% of animals. As mentioned, ifenprodil's dose–response curve was flat, with incremental doses causing limited effect. By contrast, MK-801 is neuroprotective at a dose of 1 mg/kg (Lock et al., 1997), a dose that terminated SE in 50% of the rats. In addition, animals also showed a clear response to MK-801 dose increments.

Sedation preceded successful termination of SE. This sedation was mild and following administration of the drug doses described above, stupor, coma or respiratory compromise did not occur. Sedative and other cognitive effects of NMDA receptor antagonists may deter their use in chronic epilepsy, but should have little impact on their use in the treatment of refractory SE. Patients in refractory SE are typically in stupor or coma and are unlikely to notice cognitive effects of a single dose of an NMDA receptor antagonist. Long clinical experience with ketamine anesthesia clearly demonstrates that there can be no lasting cognitive effect of a single large dose of an NMDA receptor antagonist.

The findings of the current study complement similar studies in the past that demonstrated the efficacy of NMDA receptor antagonists in terminating prolonged SE (Bertram and Lothman, 1990; Borris et al., 2000; Mazarati and Wasterlain, 1999). Initial studies on NMDA antagonist treatment of SE suggested that this class of drugs is ineffective, however these agents were administered early in the course of SE (Clifford et al., 1990; Fariello et al., 1989; Walton and Treiman, 1991). Later studies demonstrated that prolonged SE can be controlled by NMDA receptor antagonists in several animal models. Recently a commonly used anesthetic ketamine, which blocks NMDA receptors, was demonstrated to control prolonged SE in the CHS model of SE (Borris et al., 2000). There are some recent case reports of treatment of human SE with ketamine (Mewasingh et al., 2003; Ubogu et al., 2003). However, a prospective, randomized, blinded and controlled clinical trial is necessary to determine the efficacy of ketamine in the treatment of refractory SE.

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References

- Allredge BK, Gelb AM, Isaacs SM, Corry MD, Allen F, Ulrich S, Gottwald MD, O'Neil N, Neuhaus JM, Segal MR, Lowenstein DH. A comparison of lorazepam, diazepam, and placebo for the treatment of out-of-hospital status epilepticus. *N Engl J Med* 2001;345:631–637. [PubMed: 11547716]
- Beldhuis HJ, Everts HG, Van der Zee EA, Luiten PG, Bohus B. Amygdala kindling-induced seizures selectively impair spatial memory. I Behavioral characteristics and effects on hippocampal neuronal protein kinase C isoforms. *Hippocampus* 1992;2:397–409. [PubMed: 1308196]
- Bertram EH, Lothman EW. NMDA receptor antagonists and limbic status epilepticus: a comparison with standard anticonvulsants. *Epilepsy Res* 1990;5:177–184. [PubMed: 2166658]
- Borris DJ, Bertram EH, Kapur J. Ketamine controls prolonged status epilepticus. *Epilepsy Res* 2000;42:117–122. [PubMed: 11074184]
- Bouchard P, Maurice T, St Pierre S, Privat A, Quirion R. Neuropeptide Y and the calcitonin gene-related peptide attenuate learning impairments induced by MK-801 via a sigma receptor-related mechanism. *Eur J Neurosci* 1997;9:2142–2151. [PubMed: 9421174]

- Brackett RL, Pouw B, Blyden JF, Nour M, Matsumoto RR. Prevention of cocaine-induced convulsions and lethality in mice: effectiveness of targeting different sites on the NMDA receptor complex. *Neuropharmacology* 2000;39:407–418. [PubMed: 10698007]
- Clifford DB, Olney JW, Benz AM, Fuller TA, Zorumski CF. Ketamine, phencyclidine, and MK-801 protect against kainic acid-induced seizure-related brain damage. *Epilepsia* 1990;31:382–390. [PubMed: 2196169]
- Collingridge GL, Lester RA. Excitatory amino acid receptors in the vertebrate central nervous system. *Pharmacol Rev* 1989;41:143–210. [PubMed: 2558391]
- DeLorenzo RJ, Hauser WA, Towne AR, Boggs JG, Pellock JM, Penberthy L, Garnett L, Ko D. A prospective, population-based epidemiologic study of status epilepticus in Richmond, Virginia. *Neurology* 1996;46:1029–1035. [PubMed: 8780085]
- Dingledine R, Borges K, Bowie D, Traynelis SF. The glutamate receptor ion channels. *Pharmacol Rev* 1999;51:7–61. [PubMed: 10049997]
- Dogan A, Rao AM, Baskaya MK, Rao VL, Rastl J, Donaldson D, Dempsey RJ. Effects of ifenprodil, a polyamine site NMDA receptor antagonist, on reperfusion injury after transient focal cerebral ischemia. *J Neurosurg* 1997;87:921–926. [PubMed: 9384405]
- Fariello RG, Golden GT, Smith GG, Reyes PF. Potentiation of kainic acid epileptogenicity and sparing from neuronal damage by an NMDA receptor antagonist. *Epilepsy Res* 1989;3:206–213. [PubMed: 2543557]
- Friedman LK. Developmental switch in phenotypic expression of preproenkephalin mRNA and 45Ca^{2+} accumulation following kainate-induced status epilepticus. *Brain Res Dev Brain Res* 1997;101:287–293.
- Fujikawa DG. The temporal evolution of neuronal damage from pilocarpine-induced status epilepticus. *Brain Res* 1996;725:11–22. [PubMed: 8828581]
- Fujikawa DG, Daniels AH, Kim JS. The competitive NMDA receptor antagonist CGP 40116 protects against status epilepticus-induced neuronal damage. *Epilepsy Res* 1994;17:207–219. [PubMed: 7912191]
- Jones DM, Esmaeil N, Maren S, Macdonald RL. Characterization of pharmacoresistance to benzodiazepines in the rat Li-Pilocarpine model of status epilepticus. *Epilepsy Res* 2002;50:301–312. [PubMed: 12200221]
- Kapur J, Coulter DA. Experimental status epilepticus alters GABA_A receptor function in CA1 pyramidal neurons. *Ann Neurol* 1995;38:893–900. [PubMed: 8526461]
- Kapur J, Macdonald RL. Rapid seizure-induced reduction of benzodiazepine and Zn^{2+} sensitivity of hippocampal dentate granule cell GABA_A receptors. *J Neurosci* 1997;17:7532–7540. [PubMed: 9295398]
- Kohira I, Akiyama K, Daigen A, Otsuki S. Enduring increase in membrane-associated protein kinase C activity in the hippocampal-kindled rat. *Brain Res* 1992;593:82–88. [PubMed: 1458321]
- Lan JY, Skeberdis VA, Jover T, Grooms SY, Lin Y, Araneda RC, Zheng X, Bennett MV, Zukin RS. Protein kinase C modulates NMDA receptor trafficking and gating. *Nat Neurosci* 2001;4:382–390. [PubMed: 11276228]
- Lemos T, Cavalheiro EA. Status epilepticus and the late development of spontaneous seizures in the pilocarpine model of epilepsy. *Epilepsy Res* 1996;12 (Suppl):137–144.
- Lock EA, Gyte A, Duffell SJ, Simpson MG, Wyatt I. Neuroprotection afforded by MK-801 against l-2-chloropropionic acid-induced cerebellar granule cell necrosis in the rat. *Toxicology* 1997;123:41–51. [PubMed: 9347923]
- Lothman E. The biochemical basis and pathophysiology of status epilepticus. *Neurology* 1990;40:13–23. [PubMed: 2185436]
- Lothman EW, Bertram EH, Bekenstein JW, Perlin JB. Self-sustaining limbic status epilepticus induced by ‘continuous’ hippocampal stimulation: electrographic and behavioral characteristics. *Epilepsy Res* 1989;3:107–119. [PubMed: 2707248]
- Lothman EW, Perlin JB, Salerno RA. Response properties of rapidly recurring hippocampal seizures in rats. *Epilepsy Res* 1988;2:356–366. [PubMed: 3197705]
- Lurton D, Cavalheiro EA. Neuropeptide-Y immunoreactivity in the pilocarpine model of temporal lobe epilepsy. *Exp Brain Res* 1997;116:186–190. [PubMed: 9305828]

- Macdonald RL, Kelly KM. Antiepileptic drug mechanisms of action. *Epilepsia* 1995;36:S2–S12. [PubMed: 8784210]
- Mangan PS, Kapur J. Factors underlying bursting behavior in a network of cultured hippocampal neurons exposed to zero magnesium. *J Neurophysiol* 2004;91:946–957. [PubMed: 14534286]
- Mayer SA, Claassen J, Lokin J, Mendelsohn F, Dennis LJ, Fitzsimmons BF. Refractory status epilepticus: frequency, risk factors, and impact on outcome. *Arch Neurol* 2002;59:205–210. [PubMed: 11843690]
- Mazarati AM, Baldwin RA, Sankar R, Wasterlain CG. Time-dependent decrease in the effectiveness of antiepileptic drugs during the course of self-sustaining status epilepticus. *Brain Res* 1998;814:179–185. [PubMed: 9838100]
- Mazarati AM, Wasterlain CG. *N*-Methyl-D-aspartate receptor antagonists abolish the maintenance phase of self-sustaining status epilepticus in rat. *Neurosci Lett* 1999;265:187–190. [PubMed: 10327162]
- Mewasingh LD, Sekhara T, Aeby A, Christiaens FJ, Dan B. Oral ketamine in paediatric non-convulsive status epilepticus. *Seizure* 2003;12:483–489. [PubMed: 12967577]
- Meyer DA, Carta M, Partridge LD, Covey DF, Valenzuela CF. Neurosteroids enhance spontaneous glutamate release in hippocampal neurons. Possible role of metabotropic sigma1-like receptors. *J Biol Chem* 2002;277:28725–28732. [PubMed: 12042305]
- Mott DD, Doherty JJ, Zhang S, Washburn MS, Fendley MJ, Lyuboslavsky P, Traynelis SF, Dingledine R. Phenylethanamines inhibit NMDA receptors by enhancing proton inhibition. *Nat Neurosci* 1998;1:659–667. [PubMed: 10196581]
- Osonoe K, Ogata S, Iwata Y, Mori N. Kindled amygdaloid seizures in rats cause immediate and transient increase in protein kinase C activity followed by transient suppression of the activity. *Epilepsia* 1994;35:850–854. [PubMed: 8082633]
- Prasad A, Williamson JM, Bertram EH. Phenobarbital and MK-801, but not phenytoin, improve the long-term outcome of status epilepticus. *Ann Neurol* 2002;51:175–181. [PubMed: 11835373]
- Rice AC, DeLorenzo RJ. *N*-Methyl-D-aspartate receptor activation regulates refractoriness of status epilepticus to diazepam. *Neuroscience* 1999;93:117–123. [PubMed: 10430476]
- Towne AR, Pellock JM, Ko D, DeLorenzo RJ. Determinants of mortality in status epilepticus. *Epilepsia* 1994;35:27–34. [PubMed: 8112254]
- Treiman DM, Meyers PD, Walton NY, Collins JF, Colling C, Rowan AJ, Handforth A, Faught E, Calabrese VP, Uthman BM, Ramsay RE, Mamdani MB. A comparison of four treatments for generalized convulsive status epilepticus. *N Engl J Med* 1998;339:792–798. [PubMed: 9738086]
- Ubogu EE, Sagar SM, Lerner AJ, Maddux BN, Suarez JJ, Werz MA. Ketamine for refractory status epilepticus: a case of possible ketamine-induced neurotoxicity. *Epilepsy Behav* 2003;4:70–75. [PubMed: 12609230]
- Vallee M, Mayo W, Darnaudery M, Corpechot C, Young J, Koehl M, Le M, Baulieu EE, Robel P, Simon H. Neurosteroids: deficient cognitive performance in aged rats depends on low pregnenolone sulfate levels in the hippocampus. *Proc Natl Acad Sci USA* 1997;94:14865–14870. [PubMed: 9405705]
- Walton NY. Systemic effects of generalized convulsive status epilepticus. *Epilepsia* 1993;34 (Suppl 1):S54–S58. [PubMed: 8462491]
- Walton NY, Treiman DM. Response of status epilepticus induced by lithium and pilocarpine to treatment with diazepam. *Exp Neurol* 1988;101:267–275. [PubMed: 3396644]
- Walton NY, Treiman DM. Motor and electroencephalographic response of refractory experimental status epilepticus in rats to treatment with MK-801, diazepam, or MK-801 plus diazepam. *Brain Res* 1991;553:97–104. [PubMed: 1933279]
- Wang YH, Bosy TZ, Yasuda RP, Grayson DR, Vicini S, Pizzorusso T, Wolfe BB. Characterization of NMDA receptor subunit-specific antibodies: distribution of NR2A and NR2B receptor subunits in rat brain and ontogenic profile in the cerebellum. *J Neurochem* 1995;65:176–183. [PubMed: 7790859]
- Yaffe K, Lowenstein DH. Prognostic factors of pentobarbital therapy for refractory generalized status epilepticus. *Neurology* 1993;43:895–900. [PubMed: 8492944]

Fig. 1.

Dose-response curves for CPP, MK-801, and ifenprodil. The response measured was the percent fraction of animals in which SE was terminated within a period of 300 min following drug administration. (A) The fraction of rats responding to CPP increased steadily as a function of the dose and SE was controlled in all animals at a dose of 15 mg/kg. (B) The MK-801 dose-response relationship was steep, reaching a peak response of 75% at a dose of 2 mg/kg. When the dose was increased further (2.5 mg/kg), the response declined to 20%. (C) The ifenprodil dose-response curve was flat, with little increase in response as the drug dose was increased.

Fig. 2. CPP (15 mg/kg), MK-801 (2 mg/kg) and ifenprodil (30 mg/kg) were compared for effectiveness in terminating SE within 60 min (A) and 300 min (B) of drug administration.