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ORIGINAL RESEARCH ARTICLE

Antiepileptic drugs and agents that inhibit voltage-gated sodium channels prevent NMDA antagonist neurotoxicity

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N-methyl-D-aspartate (NMDA) glutamate receptor antagonists are used in clinical anesthesia and are being developed as therapeutic agents for preventing neurodegeneration in stroke, epilepsy, and brain trauma. However, the ability of these agents to produce neurotoxicity in adult rats and psychosis in adult humans compromises their clinical usefulness. In addition, an NMDA receptor hypofunction (NRHypo) state might play a role in neurodegenerative and psychotic disorders, like Alzheimer's disease, bipolar disorder and schizophrenia. Thus, developing pharmacological means of preventing these NRHypo-induced effects could have significant clinically relevant benefits. NRHypo neurotoxicity appears to be mediated by a complex disinhibition mechanism that results in the excessive stimulation of certain vulnerable neurons. Here we report our findings that five agents (phenytoin, carbamazepine, valproic acid, lamotrigine, and riluzole), thought to possess anticonvulsant activity because they inhibit voltage-gated sodium channels, prevent NRHypo neurotoxicity. The ability of tetrodotoxin, a highly selective inhibitor of voltage-gated sodium channels, to prevent the same neurotoxicity suggests that inhibition of this ion channel is the likely mechanism of action of these five agents. We also found that three other anticonvulsants (felbamate, gabapentin and ethosuximide), whose mechanism is less clear, also prevent NRHypo neurotoxicity, suggesting that inhibition of voltage-gated sodium channels is not the only mechanism via which anticonvulsants can act to prevent NRHypo neurotoxicity. Several of these agents have been found to be of clinical use in bipolar disorder. It would be of interest to determine whether these agents might have therapeutic benefits for conditions in which a NRHypo state may exist.
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Introduction

Excessive activation of N-methyl-D-aspartate (NMDA) glutamate (Glu) receptors is implicated in the pathophysiology of several neurological conditions (eg hypoxia-ischemia and seizure-mediated excitotoxic damage, neuropathic pain, and opiate dependence) and NMDA antagonists have therapeutic potential in these conditions. However, the ability of these agents to produce psychosis in humans and neurotoxicity in animals has prevented them from being used for these conditions. Developing methods for controlling or preventing these adverse reactions is an important goal, as it may permit the therapeutic potential of these agents to be realized.

There are additional reasons for studying these adverse effects of NMDA antagonists. Because the psychotic reaction triggered by these agents resembles the psychotic manifestations of idiopathic psychotic disorders,[1,2] exploring the mechanisms by which these drugs disrupt neural circuitry may shed new light on the nature of the network disturbances operative in psychotic illnesses.[3–5] NMDA receptor hypofunction (NRHypo), the condition induced in brain by NMDA antagonist drugs, is a condition that is endogenously present in the aging brain,[6] and is present to an exaggerated degree in the brains of patients with Alzheimer's disease (AD).[7] For this reason, and because of certain parallels between the pattern of neurodegeneration in AD and the pattern induced in rat brain by NMDA antagonists, it has been proposed that NRHypo may act in concert with amyloidogenic mechanisms to drive the neuropathological process in AD.[8] Finally, it is important to understand both the pathogenesis and ways of preventing NRHypo neurotoxicity, because certain drugs used in clinical anesthesia (ie ketamine and nitrous oxide (aka laughing gas)) are NMDA antagonists.

Antagonists of NMDA receptors (eg, phencyclidine

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(PCP), ketamine, MK-801, CPP, CPP-ene, nitrous oxide), when administered to adult rodents, induce reversible pathomorphological changes in pyramidal neurons of the posterior cingulate and retrosplenial cortices,[9] also known simply as the retrosplenial cortex (RSC).[10,11] These reversible changes consist of large vacuoles that have been identified ultrastructurally as swollen mitochondria and dilated saccules of endoplasmic reticulum. If NMDA receptor blockade is maintained for a prolonged interval, as occurs following a single high dose or repeated treatment with lower doses of an NMDA antagonist, neurons in the RSC and several other cerebrocortical and limbic brain regions undergo irreversible degeneration.[12–16]

Evidence[17,18] suggests that the mechanism of NRHypo neurotoxicity is indirect, and involves a polysynaptic chain of events (Figure 1) whereby blockade of NMDA receptors in multiple non-RSC brain regions eventually results in the loss of inhibition (ie disinhibition) of two key excitatory pathways (a cholinergic basal forebrain pathway and a glutamatergic thalamic pathway) that project to the RSC. This disinhibition results in the excessive release of ACh and Glu

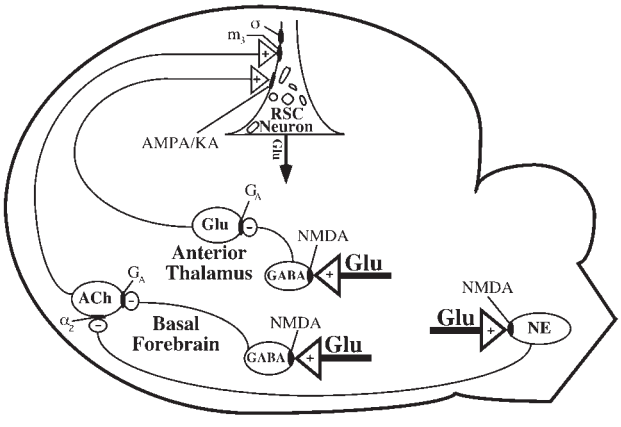

Figure 1 Circuitry proposed to mediate NRHypo neurotoxicity. To explain NRHypo neurotoxicity in the RSC, we propose that Glu acting through NMDA receptors on GABAergic and noradrenergic neurons maintains tonic inhibitory control over two major excitatory pathways that convergently innervate RSC neurons. Systemic administration of an NMDA antagonist would block NMDA receptors, thereby abolishing inhibitory control over both of the excitatory inputs to the RSC neuron. The disinhibited excitatory pathways then would simultaneously hyperactivate the RSC neuron, which would create chaotic disruption of multiple intracellular signaling systems, thereby causing immediate derangement of cognitive functions subserved by the afflicted neurons, and neuronal injury, depending on the severity of the disruption. We hypothesize that a similar disinhibition mechanism and similar but not necessarily identical neural circuits and receptor mechanisms mediate damage induced in other corticolimbic brain regions by sustained NRHypo. (+) = excitatory input; (–) = inhibitory input; ACh = acetylcholine; NE = norepinephrine; Glu = glutamate; GABA = γ-amino butyric acid; $\alpha_2 = \alpha_2$ subtype of adrenergic receptor; $G_A = GABA_A$ subtype of GABA receptor; $m_3 = m_3$ subtype of muscarinic cholinergic receptor; AMPA/KA = AMPA/KA subtype of Glu receptor; NMDA = NMDA subtype of Glu receptor; σ = sigma site.

at m_3-muscarinic and non-NMDA Glu receptors, respectively, on the injured RSC neurons. It is this simultaneous excessive stimulation of RSC neurons by these two excitatory transmitters that results in the neuronal injury.

Based upon this disinhibition model one would predict that agents that reduce the ability of these two excitatory projections to excessively release neurotransmitters and stimulate the vulnerable RSC neuron should protect against the neurotoxic reaction. Activation of voltage-gated sodium (Na^+) channels is necessary for propagation of the action potential down the axon. Inhibition of this process should prevent the synaptic release of neurotransmitter and would thus be expected to prevent NRHypo neurotoxicity. Antiepileptic drugs (AEDs) are a group of compounds that suppress neuronal firing through a broad array of mechanisms. Phenytoin and carbamazepine are two AEDs for which strong evidence exists that their basic mechanism of action is promotion of voltage-gated Na^+ channel inactivation.[19] Good evidence also exists that valproic acid and lamotrigine probably inactivate these same ion channels.[19] We, therefore, sought to determine whether these four AEDs could protect against NRHypo neurotoxicity. To develop additional evidence that inhibition of Na^+ currents is the likely mechanism of action we also studied riluzole, an agent approved for the treatment of ALS, which has been shown to also inhibit voltage-dependent Na^+ channels,[20–22] and tetrodotoxin, a poison from the puffer fish that is highly specific for voltage-gated Na^+ channels.[23,24] Finally, we were interested in whether other AEDs could also protect against NRHypo neurotoxicity. We, therefore, studied felbamate and gabapentin, two AEDs whose mechanism of action is less clear,[25] as well as ethosuximide, an AED which is not known to interact with voltage-gated Na^+ channels but instead inhibits the low threshold type of transient calcium current (T-type current).[19]

Materials and methods

Female rats are more sensitive to the neurotoxic effects of NMDA antagonists[9] probably due to differences in hepatic metabolism. We, therefore, restricted our study to this gender. Adult female Sprague–Dawley rats were injected intraperitoneally (ip) with a test agent followed 15 min later by an injection of MK-801 (0.5 mg kg^{-1}) subcutaneously (sc). At least five doses and 26 animals were studied for each agent. Phenytoin was dissolved in polyetheleneglycol (PEG). Valproic acid, gabapentin, and riluzole were dissolved in saline. Carbamazepine was dissolved in 45% HBC complex. Lamotrigine, felbamate, and ethosuximide were dissolved in dimethyl sulfoxide (DMSO). Control animals ($n = 83$) received either 45% HBC complex, PEG, DMSO, or saline, the vehicles used to dissolve the test agents, and MK-801 (0.5 mg kg^{-1} sc). Four hours after the injection of MK-801, the animals were deeply anesthetized with chloral hydrate and perfused with fixative (4% paraformaldehyde, 1.5% glutaraldehyde in

phosphate buffer at pH 7.0) through the ascending aorta. The brains were cut into 1-mm thick transverse slabs, which were then post fixed in osmium tetroxide (1%), dehydrated in a series of ethanols, cleared in toluene and embedded flat in araldite. Histological sections were cut 1 μm thick with an MT 2C Sorvall Ultratome using 0.5-inch wide glass knives. These sections were stained with a mixture of methylene blue and Azure II for light microscopic evaluation. Vacuolated RSC neurons (Figure 2a, b) were counted on each side of the brain at a level where the neurotoxic reaction is known to be near maximal (approximately 5.5 mm caudal to Bregma, a level which is easily located because it is where the corpus callosum ceases decussating across the midline,[10] Figure 2c). The investigator quantifying the reaction was blind to the treatment conditions. Percent reduction in neurotoxicity was calculated by dividing the number of vacuolated neurons in a given experimental animal by the mean number in the MK-801 controls for that AED. The result was subtracted from one and multiplied by 100. The mean number of vacuolated neurons in the control animals for each test agent varied no more than 10% from the overall mean of the 83 MK-801 controls (Figure 2d). Regression analysis was conducted with the 3-parameter sigmoid equation model of SigmaPlot (SPSS Inc, Chicago, IL, USA) in order to determine an ED_{50} (dose of a given compound that reduced the mean number of vacuolated neurons to 50% of the control mean for that agent).

For the CGS-19755 experiment CGS-19755 (20 mg kg^{-1}), a competitive NMDA antagonist, was injected into a tail vein. Phenytoin (20 mg kg^{-1}; $n = 9$ animals) or PEG ($n = 10$ animals) was subsequently

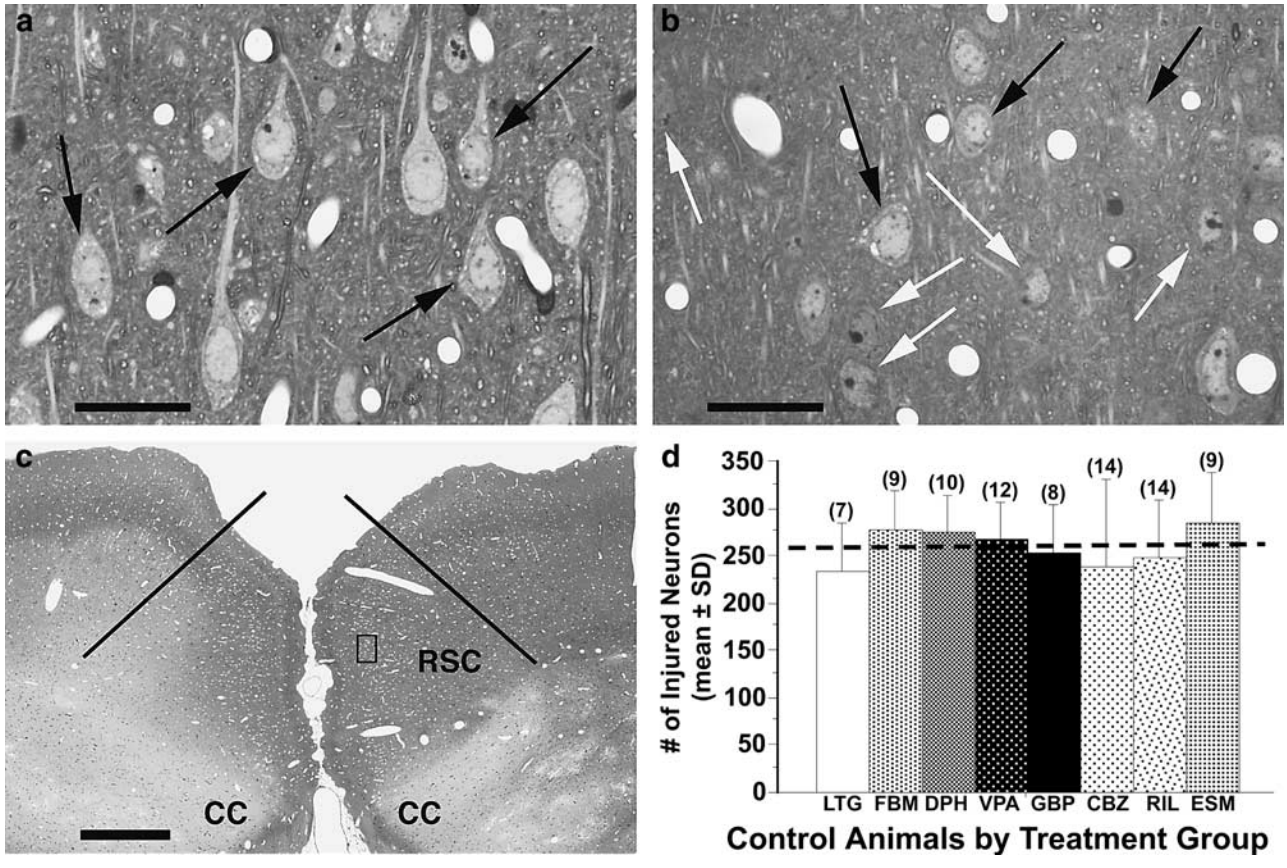

Figure 2 NRHypo neurotoxicity. (a) High power magnification of layer IV–Va pyramidal neurons from the RSC of an animal treated with MK-801, 0.5 mg kg^{-1}, sc. Almost all the pyramidal neurons in this field are injured and show the presence of vacuoles. Black scale bar = 25 μm. (b) High power magnification of layer IV–Va pyramidal neurons from the RSC of an animal treated with MK-801 (0.5 mg kg^{-1}, sc) and phenytoin (30 mg kg^{-1}, ip). Some neurons (black arrows) are injured while others (white arrows) are normal in appearance. Black scale bar = 25 μm. (c) Low power magnification of a coronal section of the cortex at approximately −5.5 mm from Bregma of an animal treated with MK-801, 0.5 mg kg^{-1}, sc. The cortex from the corpus callosum (CC) to the black line is the part of the RSC that is susceptible to NRHypo neurotoxicity. The number of injured neurons is counted bilaterally at this level in order to assess the severity of NRHypo neurotoxicity. The box indicates the area of the RSC that is shown at higher magnification in (a). Black scale bar = 1 mm. (d) Bar graph illustrating the number of injured RSC pyramidal neurons seen in the control animals ($n = 83$) in these experiments. The mean number of injured neurons seen in the control animals for each test agent did not vary by more than 10% from the overall mean for the 83 MK-801 control animals (258.5; dotted black line). Numbers in parentheses above the error bars indicate the number of control animals in each test agent group. LTG = lamotrigine, FBM = felbamate, DPH = phenytoin, VPA = valproic acid, GBP = gabapentin, CBZ = carbamazepine, RIL = riluzole, and ESM = ethosuximide.

injected intraperitoneally. Animals were killed and the tissue prepared similarly to that of the MK-801 treated animals. Statistical significance was determined by an unpaired *t*-test.

For studies involving the sterotaxic injection of tetrodotoxin directly into the RSC (AP: –5.5, ML: +0.8, DV: –1.6),[10] adult female Sprague–Dawley rats ($n = 26$) were anesthetized with isoflurane and a burr hole was made in the skull. Glass micropipettes pulled to an orifice diameter of approximately 25–50 μm were used for the microinjections. Tetrodotoxin (or saline) was injected in a volume of 0.1 μl. Immediately after the intracranial injection, the scalp wound was closed, anesthesia was discontinued and MK-801 was administered subcutaneously (sc) in a dose (0.5 mg kg^{-1}) sufficient to induce a robust vacuole reaction in RSC. When isoflurane anesthesia is terminated immediately prior to the time of MK-801 administration, it does not interfere with the neurotoxic reaction[26] because isoflurance is rapidly cleared by the lungs. Brains were processed for histological evaluation as above. Because of variation in the placement of the injection needle, counts of injured neurons were obtained from the section closest to the injection site instead of evaluating the severity of the neurotoxic reaction at a specific rostrocaudal level. An ANOVA model with one within subject independent variable (side), one between subject independent variable (dose) and one dependent measure (severity of neurotoxicity) was used to analyze the results.

Phenytoin, valproic acid, carbamazepine, riluzole, ethosuximide, tetrodotoxin and MK-801 were obtained from Sigma Chemicals (St Louis, MO, USA). Lamotrigine, gabapentin, felbamate and CGS-19755 were generously provided by Glaxowellcome (Research Triangle Park, NC, USA), Parke-Davis (Ann Arbor, MI, USA), Wallace Laboratories (Cranbury, NJ, USA), and Novartis (Summit, NJ, USA) respectively.

Results

AEDs known to inhibit voltage-gated Na+ channels

Initially we studied four AEDs, whose postulated mechanism of action is inhibition of voltage-gated Na+ channels. All four agents dose dependently prevented MK-801 neurotoxicity (Figure 3). Lamotrigine was the most potent with an ED_{50} of 8.0 mg kg^{-1}. Phenytoin and carbamazepine were less potent with ED_{50}s of 20.9 and 31.3 mg kg^{-1}, respectively. Valproic acid was roughly 10-fold less potent, having an ED_{50} of 304.3 mg kg^{-1}. These ED_{50} doses for preventing NRHypo neurotoxicity are in the same range as that for preventing maximal electroshock (MES) seizures (Table 1), consistent with the proposal that inhibition of voltage-gated Na+ channels underlies both these effects.

Other inhibitors of voltage-gated Na+ channels

To further explore whether inhibition of Na+ channels is the mechanism through which these agents prevent NRHypo neurotoxicity, we next studied riluzole, an agent not initially developed as an AED but known to block voltage-gated Na+ channels, and tetrodotoxin, a potent and selective blocker of these channels. Riluzole not only prevented the neurotoxic action of MK-801 (Figure 4) but it was more potent ($ED_{50} = 5.6$ mg kg^{-1}) than the four AEDs studied previously. Because tetrodotoxin produces paralysis of skeletal muscles and subsequent death when given systemically, it was directly injected into the RSC. It also prevented MK-801 induced neurotoxicity in RSC neurons in a dose-dependent fashion (Figure 5). When injected in a concentration of 30 nM, it provided approximately 70% blockade of the toxic reaction ipsilateral to the injection site ($P = 0.0008$).

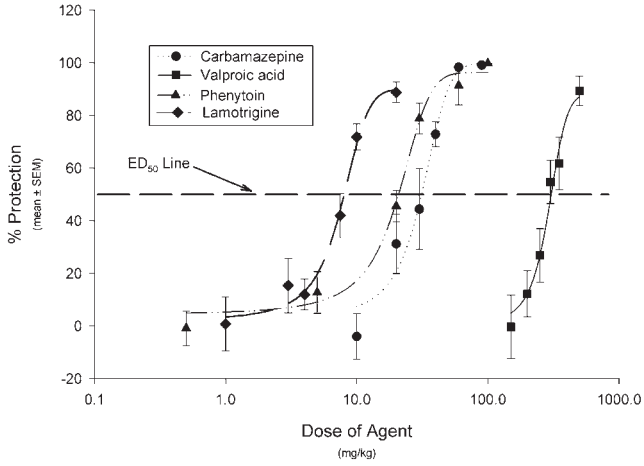

Figure 3 Ability of Na+ channel inhibiting AEDs to protect against NRHypo neurotoxicity. Lamotrigine ($n = 42$), phenytoin ($n = 45$), carbamazepine ($n = 37$), and valproic acid ($n = 34$), when administered systemically to adult rats treated with MK-801 0.5 mg kg^{-1}, sc, dose dependently prevent NRHypo neurotoxicity. Calculated ED_{50}s are 8.0 mg kg^{-1}, 20.9 mg kg^{-1}, 31.3 mg kg^{-1} and 304.3 mg kg^{-1}, respectively.

Table 1 Potency of voltage-gated Na+ channel inhibiting AEDs in preventing NRHypo neurotoxicity and MES

Voltage-gated Na+ channel inhibiting AED	*ED_{50}*	
	NRHypo neurotoxicity (mg kg^{-1})	*MES (mg kg^{-1})*
Lamotrigine	8.0	1.9[55]
Phenytoin	20.9	29.8[27]
Carbamazepine	31.3	25.9[56]
Valproic acid	304.3	490[27]

Potential site of action

We hypothesized that inhibitors of voltage gated Na+ channels would prevent NRHypo neurotoxicity because they would prevent excessive release of downstream neurotransmitters. However, it is also conceivable that these agents are effective in preventing NRHypo neurotoxicity because they prevent MK-801 from binding to the NMDA receptor. MK-801 binds to

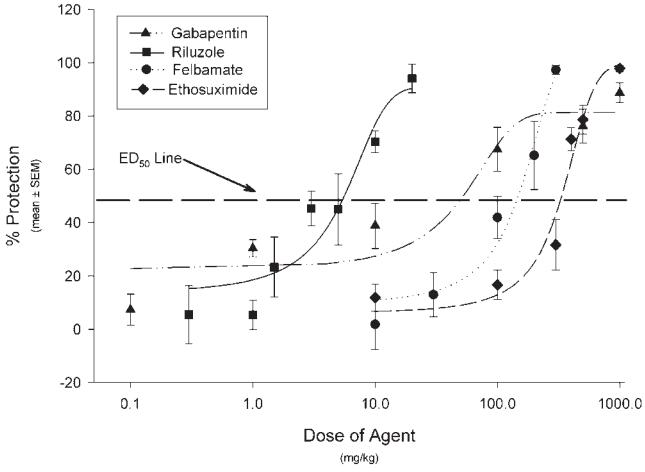

Figure 4 Ability of other agents to protect against NRHypo neurotoxicity. Riluzole ($n = 46$), gabapentin ($n = 33$), felbamate ($n = 26$), and ethosuximide ($n = 49$), when administered systemically to adult rats treated with MK-801 0.5 mg kg^{-1}, sc, dose dependently prevent NRHypo neurotoxicity. Calculated ED_{50}s are 5.6 mg kg^{-1}, 52.7 mg kg^{-1}, 148.4 mg kg^{-1} and 336 mg kg^{-1}, respectively.

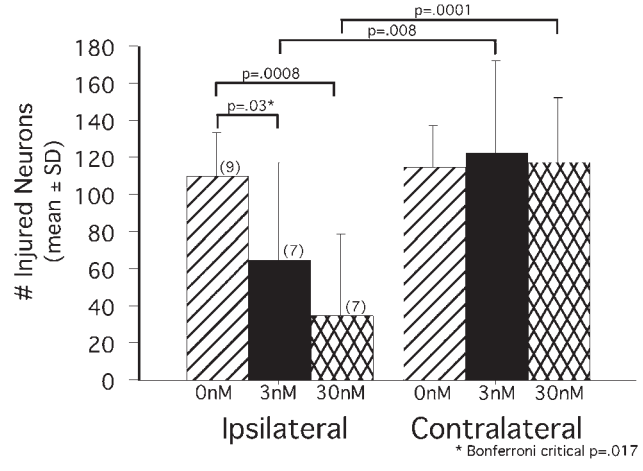

Figure 5 Ability of tetrodotoxin to protect against NRHypo neurotoxicity. Bar graph illustrating the amount of protection against NRHypo neurotoxicity provided ipsilaterally by the injection of tetrodotoxin provided directly into the RSC of animals treated with MK-801 0.5 mg kg^{-1}, sc. Compared to saline, tetrodotoxin protection against NRHypo neurotoxicity (treatment condition × side: $F(2,23) = 13.50$, $P = 0.0001$; treatment condition on ipsilateral side: $F(2,23) = 7.65$; $P = 0.003$); treatment condition on contralateral side: $F(2,23) = 0.10$; $P = 0.9$). The significant effect of treatment condition on the ipsilateral side was explained by the 30 nM condition being significantly lower than the saline ($P = 0.0008$). While the 3 nM condition also appeared to have significantly less injured neurons than the saline condition ($P = 0.03$), the result was not significant once the Bonferroni correction was applied (Bonferroni critical $P = 0.05/3 = 0.017$). For both experimental conditions the amount of damage on the ipsilateral side was significantly less than that on the contralateral side ($P = 0.008$ for 3 nM and $P = 0.001$ for 30 nM). No ipsilateral-contralateral difference was seen in the control animals ($P = 0.4$). Number in parenthesis above each bar graph indicates the number of animals in each condition.

a site inside the ion channel of the NMDA receptor complex. It can only enter the channel and bind to this site after Glu has bound to the Glu recognition site and opened the channel. Thus, by preventing the normal release of Glu at NMDA receptors, inhibitors of voltage gated Na$^+$ channels could be preventing MK-801 from binding to the NMDA receptor. In order to rule out this possibility we decided to test whether phenytoin could prevent NRHypo neurotoxicity induced by CGS-19755, a competitive NMDA antagonist. Since CGS-19755 binds to the Glu recognition site and is not dependent on channel opening for binding, it inhibits the receptor even in the situation of decreased synaptic Glu. Phenytoin (20 mg kg^{-1}, ip) significantly prevented NRHypo neurotoxicity ($P = 0.01$) induced by CGS-19755 (20 mg kg^{-1}, iv) consistent with the proposal that voltage-gated Na$^+$ channel inhibitors prevent NRHypo neurotoxicity by reducing the excessive stimulation of the vulnerable RSC neurons.

Other AEDs

Next we examined gabapentin and felbamate, two AEDs that are active in the MES seizure model but whose mechanism of action is less clear. While gabapentin's calculated ED_{50} (52.7 mg kg^{-1}) suggested that it was more potent than felbamate ($ED_{50} = 148.4$), its dose response slope was relatively shallow resulting in felbamate being more potent than gabapentin at higher doses (Figure 4). Next, we decided to examine the ED_{50}s of these two agents in both the NRHypo neurotoxicity and MES seizure models and to compare the ratio of their potencies in these models to that of the four previously studied voltage-gated Na$^+$ channel inhibiting AEDs. To accomplish this analysis we first plotted ratios of the two ED_{50}s for the four initially studied AEDs and determined the regression line formed by these ratios. Then, we plotted the values for gabapentin and felbamate. The ratio for gabapentin was similar to that of the four AEDs initially studied (Figure 6). In contrast, the ratio for felbamate was markedly different from the first four AEDs studied (Figure 6) with it being substantially weaker at preventing NRHypo neurotoxicity than one would have predicted based upon its ED_{50} in the MES seizure paradigm.

Finally, we examined ethosuximide because, while it is an anticonvulsant, it does not inhibit voltage-gated Na$^+$ channels. Ethosuximide was effective in preventing NRHypo neurotoxicity (Figure 4), but its ED_{50} of 336 mg kg^{-1} was 10-fold greater than that reported to be needed to treat pentylenetetrazol-induced seizures.[27] We could not carry out an analysis of its ED_{50} ratios because ethosuximide is inactive in the MES seizure model.

Discussion

Here we report that four AEDs, phenytoin, valproic acid, carbamazepine, and lamotrigine, can prevent the neurotoxic effects of the potent NMDA antagonist, MK-801. Furthermore their potencies at preventing this neurotoxicity are similar to their potencies in the MES

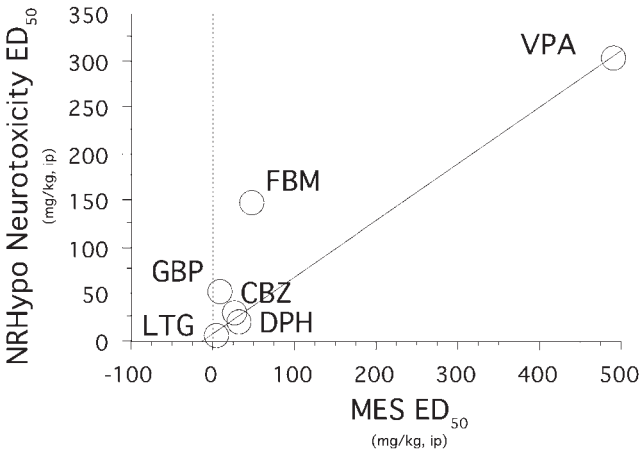

Figure 6 Comparison of AEDs' ED_{50}s for preventing NRHypo neurotoxicity and MES seizures. Plot of the ED_{50}s of several AEDs in preventing MES seizures and NRHypo neurotoxicity in rats. The linear regression line is based on the values for the four known voltage-gated Na^+ channel inhibiting AEDs (lamotrigine (LTG), phenytoin (DPH), carbamazepine (CBZ) and valproic acid (VPA)). Gabapentin (GBP) is slightly off the regression line and felbamate (FBM) is markedly off the regression line. ED_{50} values for preventing MES seizures were obtained from the literature.[27,37,55,56]

seizure paradigm, a commonly used model for assessing anticonvulsant activity. These data suggest that the mechanism of action of these agents in preventing seizures (eg inhibition of voltage-gated Na^+ channels) probably underlies their ability to prevent NRHypo neurotoxicity. The ability of tetrodotoxin, a highly selective inhibitor of voltage-gated Na^+ channel, to prevent the neurotoxicity, confirms that inactivation of this ion channel is a mechanism through which these AEDs can prevent NRHypo neurotoxicity. Riluzole, an inhibitor of voltage-gated Na^+ channels, while not initially developed as an AED, has been found to be a relatively potent anticonvulsant in the MES seizure model.[28] Because the MES study with riluzole was done in mice and not rats we were not able to directly compare its MES seizure activity against its protective activity vis-à-vis NRHypo neurotoxicity. Allowing for inter-species differences in pharmacokinetics, its potency against MES seizures in the mouse ($ED_{50} = 8.5$ mg kg^{-1}) is similar to its ED_{50} against NRHypo neurotoxicity in the rat, suggesting that the same mechanism, namely inhibition of voltage-gated Na^+ channels, may underlie its activity in both models. While these data indicate that inhibition of voltage-gated Na^+ channels is the likely mechanism through which these agents prevent NRHypo neurotoxicity, it is possible that activity at some other site could also offer some protection.

Two less well understood AEDs, gabapentin and felbamate, also prevent NRHypo neurotoxicity. The finding that felbamate is markedly less potent in protecting against NRHypo neurotoxicity than predicted, based on its reported activity in the MES seizure model, suggests that the mechanism of action is not identical in the two model systems. We believe that in the MES seizure model felbamate may be acting through at least two separate mechanisms of action, namely inhibition of Na^+ currents[29,30] and inhibition of NMDA receptors.[29,31,32] While blockade of NMDA receptors can inhibit MES seizures[33–36] such activity at the NMDA receptor would not be expected to provide protection against NRHypo neurotoxicity, since blockade of this receptor is what produces the damage. Thus, felbamate would prevent MES seizures through two mechanisms while preventing NRHypo neurotoxicity through only one mechanism, resulting in felbamate being less potent against NRHypo neurotoxicity than predicted by its activity in the MES seizure model.

The mechanism underlying gabapentin's ability to prevent NRHypo neurotoxicity is unclear. The ratio of its potencies in the MES seizure and the NRHypo neurotoxicity models was close to that seen with the four AEDs initially studied, suggesting that a similar mechanism might underlie both its effects. There is no good evidence that gabapentin inhibits voltage-gated Na^+ channels. Instead investigators suspect that its main mode of action is through increasing GABA release.[19,37] Increasing GABA release could be a potential mechanism through which gabapentin prevents NRHypo neurotoxicity because a wide range of GABAergic agents are known to prevent NRHypo neurotoxicity.[13,26,38]

While ethosuximide does prevent NRHypo neurotoxicity, it does so at a dose ($ED_{50} = 336$ mg kg^{-1}) that is approximately 10-fold greater than that needed to prevent pentylenetetrazol-induced seizures (ED_{50} = 54 mg kg^{-1}),[27] suggesting that its mechanism of action is different in the two model systems. Since its suspected mechanism of action against pentylenetetrazol-induced seizures is inhibition of T-type calcium currents,[19] we tentatively conclude that inhibition of this type of calcium current is not an efficacious method of preventing NRHypo neurotoxicity. However, investigation with selective inhibitors of T-type calcium currents will be needed in order to more definitively rule out this possibility. Thus, the mechanism through which ethosuximide is preventing NRHypo neurotoxicity remains unknown. However, its ability to prevent NRHypo neurotoxicity at high doses is consistent with its lack of activity in the MES seizure model and consistent with our main conclusion that inhibition of voltage-gated Na^+ channels prevents NRHypo neurotoxicity.

Based upon several similarities between the ability of NMDA antagonists to produce neurotoxicity and psychosis, it has been proposed that the disinhibitory mechanism that underlies their neurotoxic action is also responsible for producing psychosis.[3,4,39] As has been discussed elsewhere the proposal that a similar mechanism underlies both effects does not mean that the neurotoxicity is directly responsible for the psychosis.[4,39] Instead it has been proposed that mild degrees of NRHypo would produce cognitive and behavioral changes while severe degrees of NRHypo would be needed to produce neurotoxicity. Consistent

with the proposal that a similar mechanism underlies both these NRHypo effects, lamotrigine has been found to prevent ketamine-induced symptoms in human volunteers.[5] Thus, we propose that inhibition of voltage-gated Na^+ channels is one mechanism by which lamotrigine could be preventing NRHypo psychosis. To our knowledge none of the other agents that we found to be active in this series of experiments have been examined in humans for their ability to prevent NRHypo psychosis. However, we would not expect a perfect correlation between animal and human studies because such studies are difficult to conduct and interpret. As we have shown in this study, all these agents prevent NRHypo neurotoxicity in a dose-dependent fashion. We would assume that their ability to alleviate the mental effects of NRHypo in humans would also be dose-dependent. Thus, an agent at one dose might only ameliorate but not completely eliminate the NRHypo effect under study. Increasing the dose of the protective agent should result in a greater effect. However, the protective agent at higher doses might have additional actions that result, for example, in the agent adversely affecting cognition or producing sedation. While we did not rigorously study the cognitive and sedative effects of these agents, our impression was that, at the higher doses needed to obtain full protection, the animals tended to be sedated and recumbent. Thus, it is possible that an agent like phenytoin might completely alleviate the mental effects of NRHypo but only at a dose that produces a large amount of sedation, resulting in an inability to detect the improvement on examination.

While the link between NRHypo neurotoxicity and NRHypo psychosis is becoming better understood, the relationship of these two phenomena to both AD and idiopathic psychotic disorders like schizophrenia and bipolar disorder is less well understood. Directly extrapolating from a drug-induced model in normal brain to a disease process in abnormal brain is inherently difficult, especially when the disease process has a strong genetic component and the exact pathophysiological basis of the disease process is still unclear. Thus, one should not expect a one-to-one relationship between the clinical usefulness of potential therapeutic agents in the NRHypo model and clinical disease states. However, results from this type of study could indicate agents that might be more likely to be effective. To our knowledge none of these agents have been shown to be efficacious in schizophrenia. For AD we are aware of only one study. In this small study lamotrigine was found to improve cognition in AD.[40] For bipolar disorder the data are more robust. Lamotrigine,[41–43] carbamazepine,[44–47] and valproic acid[48–50] all have been shown to one degree or another to be of therapeutic value in bipolar disorder. While phenytoin has not been studied as extensively, one recent study did find it effective in bipolar disorder.[51] Thus, it appears that inhibition of voltage-gated Na^+ channels might be an effective method for treating bipolar disorder and preventing NRHypo psychoneurotoxicity. Gabapentin, an agent not known to act through inhibition of voltage-gated Na^+ channels, also prevents NRHypo neurotoxicity and early evidence is suggestive that it could be an effective treatment for bipolar disorder.[52–54] Thus, it appears that the activity in the NRHypo neurotoxicity model is predictive of whether an AED, regardless of its mechanism of action, might be an effective treatment for bipolar disorder. It might be of interest to determine whether the other agents found to prevent NRHypo neurotoxicity are also effective treatments for bipolar disorder.

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