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SHORT COMMUNICATION

NMDA NR2B subtype-selective receptor antagonists fail to antagonize electrically-precipitated seizures and elicit popping in mice

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Abstract

NR2B-subtype-selective antagonists differ from MK-801, a nonselective NMDA receptor antagonist. MK-801 antagonizes electrical seizures at doses as low as 0.1 to 0.18 mg/kg and elicits popping at doses as low as 0.5 mg/kg, whereas ifenprodil and Ro 8-4304 were unable to do so at the doses tested. Ro 25-6981, however, was able to antagonize electrically-precipitated tonic hindlimb extension at 100 mg/kg, but was not able to elicit popping behavior at this dose.

MK-801 (dizocilpine), a noncompetitive NMDA receptor antagonist, antagonizes electrically-precipitated tonic hindlimb extension and elicits irregular episodes of intense jumping behavior, referred to as "popping," in a dose-dependent manner in mice (Deutsch and Hitri, 1993; Deutsch et al., 1996, 1997a,b, 1999; Norris et al., 1992; Rosse et al., 1995). These abilities of MK-801 are modulated by glycinergic interventions that bind to the strychnine-insensitive glycine binding site on the NMDA receptor (Deutsch et al., 1999). Behavioral effects of MK-801 are most likely mediated by its binding to a hydrophobic domain within the open channel of the NMDA receptor. Although phencyclidine, ketamine and memantine, a series of "nonselective" noncompetitive NMDA receptor antagonists, shared this ability to antagonize electrical seizures with MK-801, they differed from each other in terms of their sensitivity to a stress-induced reduction of antiseizure efficacy (Deutsch et al., 1997a). Specifically, 24 h after an outbred strain of mouse was subjected to a single 10-minute session of forced swimming at 6 °C, the antiseizure efficacies of MK-801 and memantine were significantly reduced, whereas there was

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no stress-induced reduction in the antiseizure efficacies of phencyclidine and ketamine (Deutsch et al., 1997a).

The NMDA receptor is composed of individual polypeptide subunits; gating and channel properties are determined by combinatorial diversity of expressed subunits (Perera et al., 2008). These functional multimeric glutamate-gated cation channel receptors are composed of subunits from two families: one of eight splice variants of NR1 and NR2A-D, the latter are encoded by discrete genes and possess high sequence homology (Perera et al., 2008; Gogas, 2006; Mutel et al., 1998). Moreover, the patterns of subunit expression differ according to brain region and stage of development (Perera et al., 2008). The NR2 class of subunit is responsible for glutamate binding and determines synaptic localization (Gogas, 2006). The expression and clustering of the NR2B subunit on the surface of the synapse is influenced by the extent of casein kinase II-mediated phosphorylation of a specific serine residue in the C-terminal tail; additionally, enzyme-mediated tyrosine phosphorylations (e.g., calcium/calmodulin-dependent protein kinase II) of the NR2B subunit influence the function of the NMDA receptor (Gogas, 2006). Many NR2B-subunit selective antagonists are open-channel blockers, whose effectiveness depend on channel opening, and may interact allosterically with other ligand binding sites (e.g., those for polyamines) (Gogas, 2006).

Because nonselective NMDA receptor antagonists disrupt normal processes of cognition and memory and may precipitate psychosis, their development as medications for neuroprotection, seizures and other indications is limited (Gogas, 2006). However, memantine was developed and approved for an indication in Alzheimer's disease. Ideally, highly-selective receptor-subtype specific antagonists that bind to specific subunits or subunit combinations would avoid undesired side effects. Thus, we wondered if several characterized NR2B receptor-subtype-selective NMDA antagonists shared MK-801's ability to antagonize electrically-precipitated tonic hindlimb extension and elicit popping behavior. Behavioral testing with relevant paradigms could identify candidate compounds for development as medications for chronic administration (e.g., to treat seizures), while minimizing or avoiding undesired side effects (e.g., disruption of cognition and psychosis). Specifically, we tested ifenprodil ((1R*,2S*)-erythro-2-(4-benzylpiperidino)-1-(4-hydroxyphe nyl)-1-propanol hemitartrate), Ro 25-6981 ((aR,bS)-a-(4-hydroxyphenyl)-b-methyl-4-(phenylmethyl)-1-piperidinepropanol maleate), and Ro 8-4304 (4-{3-[4-(4-fluoro-phenyl)-3,6-dihydro-2H-pyridin-1-yl]-2-hydroxy-propoxy}-benzamide), a series of NR2B-subtype-selective NMDA receptor antagonists, for their abilities to antagonize electrical seizures and elicit popping in male outbred NIH Swiss mice.

1. Experimental procedures

1.1. Subjects

Groups of 5 experimentally-naïve, age-matched, male outbred NIH Swiss mice (Hilltop Laboratories, Scottdale, PA), approximately 5 weeks of age, weighing approximately 25 g, were housed in hanging clear Plexiglas cages with free access to food and water, and maintained on a 12 h light/dark cycle. Mice were weighed individually prior to drug administration and 12–17 mice were tested in each condition for the incremental electroconvulsive shock IECS procedure and 10–13 mice per group for the popping procedure.

1.2. Drugs

Ifenprodil, Ro 25-6981 (Tocris Cookson; Ellisville, MO), and Ro 8-4304 (Sigma Chemical Co.; St. Louis, MO) were dissolved in 0.9% saline and prepared on the day of the experiment. All drugs were injected intraperitoneally in a volume of 0.01 ml/g of body weight. Individual mice received saline or one of several doses of each NR2B-subtype-selective antagonist (10.0, 32.0, and 100.0 mg/kg ip) 20 min before testing in the incremental electroconvulsive shock (IECS) procedure. Individual mice received saline or 100.0 mg/kg of ifenprodil, Ro 25-6981, or Ro 8-4304 prior to visual observation of popping episodes.

1.3. Incremental electroconvulsive shock (IECS) procedure

In the IECS procedure, a Hittman electroconvulsive shock generator (Medcraft model B24-III) is utilized to administer 0.3 s of voltage via earclip electrodes. To determine threshold voltages for the precipitation of tonic hindlimb extension, the procedure begins with 70 V and is increased in 10 V increments every 2 s until the maximal tonic hindlimb extension occurs or 170 V is reached. A voltage of 180 is recorded for animals that do not show tonic hindlimb extension.

1.4. Behavioral observation of episodic popping behavior

Individual mice are placed in clear plastic cages (28×20×15 cm) with crushed corn-cob as bedding for behavioral observation. Discrete episodes of "popping" are counted for the entire interval between 5 and 60 min following intraperitoneal injection. Although the episodes could differ from each other with respect to duration and intensity, discrete episodes of popping can be reliably observed and recorded (Deutsch and Hitri, 1993). Group sizes of no more than 10 animals are observed simultaneously.

These studies were approved by the Institutional Animal Care and Use Committee and conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

2. Results

Although all of the data for the IECS procedure are presented in Fig. 1, one-way analyses of variance were performed separately for each of the NR2B-subtype-selective antagonists. With respect to ifenprodil and Ro 8-4304, ANOVAs were not significant, suggesting that these NR2B-subtype-selective compounds at doses of 10.0, 32.0 and 100 mg/kg were not able to raise threshold voltages for precipitation of tonic hindlimb extension. However, a one-way ANOVA did show a significant main effect for dose of Ro 25-6981 in the IECS procedure ($F_{3,56}=6.239$, $p=0.001$). Visual inspection of the graphed data suggests that the 100 mg/kg dose affords minimal protection against electrically-precipitated seizures. Thus, "exploratory" individual post hoc least significant differences (LSD) tests were performed comparing the 100 mg/kg dose of Ro 25-6981 with each of its lower doses (vs 10.0 mg/kg, LSD test, $p=0.013$; vs 32.0 mg/kg, LSD test, $p=0.001$) and placebo (LSD test, $p<0.001$); all of these individual post hoc comparisons were significant, supporting the possibility that the 100 mg/kg dose of Ro 25-6981 affords minimal protection against electrically-precipitated seizures. Nonetheless, even if minimal protection is afforded by Ro 25-6981, MK-801 would still be about 500- to