Available online at www.sciencedirect.com
ScienceDirect
Neuropharmacology 53 (2007) 821–831
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

Two-methyl-6-phenylethynyl-pyridine (MPEP), a metabotropic glutamate receptor 5 antagonist, with low doses of MK801 and diazepam: A novel approach for controlling status epilepticus

Feng Ru Tang [a,b,*], Peng Min Chen [a], Yong Cheng Tang [a], Mui Chiung Tsai [a], Wei Ling Lee [c]

[a] *Epilepsy Research Lab, National Neuroscience Institute, 11 Jalan Tan Tock Seng, Singapore 308433*
[b] *Department of Anatomy, National University of Singapore, Singapore 117597*
[c] *Department of Neurology, National Neuroscience Institute, 11 Jalan Tan Tock Seng, Singapore 308433*

Received 23 March 2007; received in revised form 6 August 2007; accepted 8 August 2007

Abstract

By intravenous administration of group I metabotropic glutamate receptor antagonists at 1 or 2 h during pilocarpine induced status epilepticus (PISE), we showed that mGluR1 antagonists AIDA or LY367385 (at dosages ranging from 25 to 200 mg/kg), mGluR5 antagonists SIB1757 (at dosages ranging from 25 to 200 mg/kg), SIB1893 (from 25 to 100 mg/kg), MPEP (from 25 to 100 mg/kg) injected at 1 or 2 h during PISE were ineffective in controlling status epilepticus (SE). However, when administered at 1 h during PISE, MPEP at 200 mg/kg, combination of MPEP (200 mg/kg) with MK801 (0.1 mg/kg) or with MK801 (0.1 mg/kg) and diazepam (0.5 mg/kg), combination of SIB1893 (200 mg/kg) with MK801 (0.1 mg/kg) could effectively control behavioral SE, and were neuroprotective. In particular, the combination of MPEP with MK801 and diazepam could stop both behavioral SE and electrical SE (under EEG monitoring) within a few minutes after the administration. HPLC study showed that a high level of MPEP was maintained in the blood and its metabolism rate was slow in experimental mice with PISE. We therefore concluded that the combination of MPEP (200 mg/kg) with MK801 (0.1 mg/kg) and diazepam (0.5 mg/kg) could effectively stop SE and its subsequent neuronal loss in the hippocampus when administered 1 h during PISE. It may provide a new approach to effectively control intractable SE.

Keywords: Group I metabotropic glutamate receptor; MPEP; Status epilepticus; Neuroprotection

1. Introduction

Status epilepticus (SE) is a neurological emergency with risk of mortality (Hesdorffer et al., 1998) or serious neurological sequelae such as focal neurological deficits, intellectual deterioration, and perhaps chronic epilepsy (Shorvon, 1993). Hence immediate and effective control of SE is necessary. However, current first-line anticonvulsants often fail to terminate SE and about 30–50% of patients have refractory SE (Treiman et al., 1998). Therefore, novel ideas and new approaches are needed. We hypothesized that combination of high dosages of antagonists of group I metabotropic glutamate receptors (mGluRs) (to modulate synaptic hyperactivity) with low dosages of antagonists of ionotropic glutamate receptors (iGluRs) (particularly NMDA receptor, to mediate synergic interactions) and diazepam (to enhance GABA mediated inhibitory neurotransmission) at early stages after SE, might not only effectively control SE, but also prevent its subsequent neuronal loss. We are especially interested in metabotropic glutamate receptors, because they are located at the periphery of synapse, and ''modulate'' rather than ''mediate'' excitatory

* Corresponding author. Epilepsy Research Lab, National Neuroscience Institute, 11 Jalan Tan Tock Seng, Singapore 308433. Tel.: +65 63577128; fax: +65 62569178.
E-mail address: feng_ru_tang@nni.com.sg (F.R. Tang).

0028-3908/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2007.08.012

synaptic transmission under particularly circumstances such as synaptic hyperactivity (as seen in epilepsy), and are rarely present in target organs of the autonomic nervous system, and therefore, have been considered as novel targets in the treatment of epilepsy (Nicoletti et al., 1996). In the present study, we aimed to: (1) find out if pilocarpine induced status epilepticus (PISE), and its subsequent neuronal loss in the hilus of the dentate gyrus could be effectively controlled after systemic administration of selective mGluR1 antagonists AIDA, LY367385, mGluR5 antagonists 2-methyl-6-phenylethynyl-pyridine (MPEP), SIB1757, SIB1893, NMDA receptor antagonist MK801, $GABA_A$ receptor agonist diazepam, at different dosages at 1 or 2 h during PISE; (2) determine if combination of MPEP with lower dosages of NMDA receptor antagonist MK801, and/or $GABA_A$ receptor agonist diazepam, could more effectively control intractable SE and subsequent neuronal loss than MPEP alone; (3) evaluate the plasma profile of an effective drug, MPEP, and its long-term effect on main organs such as heart, liver, lung, kidney and spleen by pharmacokinetic and pharmacodynamic studies as similar study has not been reported according to our knowledge. We are particularly interested in the combination of MPEP with MK801 and diazepam, because it has been known that MPEP blocks convulsive and non-convulsive primary generalized seizures in DBA/2 mice and lethargic mice (Chapman et al., 2000), stops seizures induced by lower doses of pentylenetetrazole (PTZ) (Nagaraja et al., 2004), and prevents neuronal damage (Bruno et al., 2001). MK801 or diazepam at high doses can control seizures, but is associated with unacceptable side effects in clinical trial or practice. Furthermore, activation of mGluR5 potentiates or depresses AMPA (Ugolini et al., 1999), NMDA (Awad et al., 2000), mGluR1 (Lanneau et al., 2002) or GABA receptors (Besheer and Hodge, 2005) and modulates the activity of voltage-operated Ca^{2+} channels (VOCC) (Sanchez-Prieto et al., 2004). By systemic administration of MPEP, we hoped that it could not only antagonize the activity of mGluR5 per se, but also modulate NMDAR, mGluR1 and GABA receptors, and produce synergic anticonvulsive and neuroprotective effects with minimal side effect, so that we could test a new strategy to reduce SE by combining mGluR5 antagonists with low doses of diazepam and MK801.

2. Materials and methods

2.1. Intravenous cannulation, induction of SE, drug administration and cresyl fast violet (CFV) histochemistry

Male Swiss mice weighing 25–30 g (2-month-old) were used in this study. Intravenous cannulation (with polythene tubing: OD, 0.45 mm; ID, 0.30 mm; Science Team Services, Singapore) was done through the jugular vein in chloral hydrate (0.40 g/kg) anaesthetized mice. Mice recovered at about 2 h after operation. Three days after cannulation, mice were given a single subcutaneous injection of methyl-scopolamine nitrate (Sigma; 1 mg/kg) 30 min before the injection of pilocarpine to limit the peripheral toxic effects of the latter (Tang et al., 2005, 2006). They then received a single i.p. injection of pilocarpine (300 mg/kg) and experienced behavioral changes including hypoactivity, tremor, mild facial clonus, head bobbing, and myoclonic movements of the limbs progressing to recurrent myoclonic convulsions with rearing, falling and status epilepticus. The convulsive behavior was scored according to Racine (1972) where 0, no reaction; 1, stereotype mouthing, eye blinking and/or mild facial clonus; 2, head nodding and/or severe facial clonus; 3, myoclonic jerks in the forelimbs, and running; 4, clonic convulsions in the forelimbs with rearing and 5, generalized clonic convulsions associated with loss of balance. One or 2 h during PISE, drugs or saline were administered intravenously to experimental or control mice. All experiments were approved by the Institutional Animal Care & Use Committee of the Tan Tock Seng Hospital – National Neuroscience Institute. In the handling and care of all animals, the guidelines for animal research of NIH were strictly followed. Efforts were made throughout the study to minimize animal suffering and to use the minimum number of animals.

To evaluate anticonvulsive and neuroprotective effect of drugs, normal control mice with saline injection (without pilocarpine induced SE), control mice with SE (induced by pilocarpine) treated with saline 1 h during PISE were used for comparison with mice treated by drugs at 1 or 2 h during PISE (refer to Tables 1, 2 and 3). In order to exclude variations caused by intraperitoneal (i.p.) injection of a very small volume of drugs, we cannulated all mice, and did intravenous administration (through jugular vein) for all the drugs. All the single drugs were administered in about 3 min, and the combination of two drugs was given within 4 min, and of three drugs in 5 min. The behavioral changes after drug or saline injection during PISE were monitored for about 4 h by the experimenter. Mice were killed 24 h after PISE, and the brain was removed and kept in 10% formalin, then embedded with paraffin. Serial sections with the thickness of 5 μm were cut with microtome and stained with CFV. The brain of normal control mice with saline injection (without SE) was also prepared 24 h later. Six sections of the dorsal hippocampus (one in every five sections, from 2.30 to 2.46 mm posterior to bregma) from each mouse were selected for quantitative analysis, and the number of neurons in the hilus of the dentate gyrus was counted using KS 100 Imaging System, and scored as number of neurons/per mm^2. In the present study, quantitative study of CA1 area was not done because our previous study showed that in CA1 and CA3 areas, neuronal loss was obvious only at 3 days, but not 1 day after PISE, however, drastic loss of hilar neurons occurred at the later time point (Tang et al., 2005, 2006). The area of the hilus was defined by an encirclement artificially drawn along the subgranular layer of the dentate gyrus with the two ends of the line in the upper and lower blades being connected by a straight line. For cell counting, subgranular cells, one cell layer inside the stratum granulosum, and CA3c pyramidal cells in the triangle were not included. Data from the experimental and control groups were analyzed using One way ANOVA with Dunnett's test.

2.2. Video camera and electroencephalography (EEG) monitoring

To further evaluate if drugs could effectively control SE at both behavioral and EEG levels, video camera and EEG monitoring were done simultaneously in 42 mice (Table 4). For EEG monitoring, a transmitter (TSE, Bad Homburg, Germany) was fixed on the electrode socket by plug connection with wires attached to the mouse skull by two screws (2.3 mm posterior and 2 mm lateral to bregma) 3 days before pilocarpine injection. The EEG signals were telemetrically received via HF receiver which passed the signals onto the computer. Special telemetry interfaces built into the computer decoded and processed the signals from the receiver and then transferred them to the personal computer. Signals were then read by the TSE TeleSys data acquisition and analysis program. SE duration was defined as the time period during which the EEG spiking exhibited a primary frequency of 1 Hz, and behavioral change was above stage 3. Video camera recording was done simultaneously, so as to correlate behavioral changes to EEG data. Animals were killed 24 h after treatment with MPEP or its combinations (refer to Table 4), and the brains were removed and processed for paraffin embedding and CFV staining as stated above. Twelve SE mice (three for each drug) were treated with LY367585, AIDA, SIB1757 and SIB1893 at 200 mg/kg, and monitored for EEG changes.

2.3. Hematoxylin/eosin (H and E) staining to evaluate toxic effect of MPEP + MK801 + diazepam on cardiac muscle, liver, lung, kidney, and spleen

To evaluate if a combination of MPEP (200 mg/kg) with MK801 (0.1 mg/kg) and diazepam (0.5 mg/kg) produces any toxic effect or damage on heart,