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Capacities of metabotropic glutamate modulators in counteracting soman-induced seizures in rats

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ABSTRACT

Current treatment of nerve agent poisoning with ionotropic drugs proves inadequate, and alternative treatment strategies are searched for. Based on positive findings with metabotropic glutamate modulators in microinfusion studies, the present study was initiated to examine anticonvulsant effects of MPEP (2-Methyl-6-(phenylethynyl)pyridine hydrochloride), a metabotropic glutamate receptor 5 antagonist, and DCG-IV ((2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine), a metabotropic glutamate receptor 2/3 agonist, when administered systemically in combinations with HI-6 (1-[([4-(aminocarbonyl) pyridino]methoxy)methyl]-2-[(hydroxyimino)methyl]pyridinium) and procyclidine or HI-6 and levetiracetam relative to the combination of HI-6, procyclidine, and levetiracetam. The results showed that MPEP or DCG-IV combined with HI-6 and procyclidine resulted in substantial antidotal efficacy when administered 20 min after onset of seizures elicited by soman. MPEP or DCG-IV combined with HI-6 and levetiracetam did not terminate seizures and preserve lives. When given 20 min before challenge with soman, DCG-IV in combination with HI-6 and procyclidine provided protection, whereas MPEP combined with HI-6 and procyclidine did not. Combinations with metabotropic glutamate receptor modulators did not achieve the same high level of antidotal efficacy as the combination of HI-6, procyclidine, and levetiracetam. MPEP alone inhibited pseudocholinesterase activity in the brain markedly. A positive correlation was found between latency to seizure onset or full protection and level of pseudocholinesterase activity in brain. MPEP and DCG-IV can serve as effective anticonvulsants against nerve agent poisoning when combined with HI-6 and procyclidine. Metabotropic glutamate receptor modulators may represent an alternative or supplement to treatment with ionotropic drugs.

1. Introduction

Organophosphorus nerve agents are highly potent irreversible inhibitors of the enzyme acetylcholinesterase that hydrolyzes acetylcholine. Accumulation of acetylcholine in the synaptic cleft results in over-stimulation of muscarinic and nicotinic receptors. It has been hypothesized that several neurotransmitter systems become involved sequentially in the initiation and maintenance of seizures elicited by nerve agents (McDonough and Shih, 1997). The progression of events can conceptually be divided into 3 phases. An early cholinergic phase lasting from the time of exposure to about 5 min after onset of seizures is dominated by high cholinergic activity followed by a transitional phase of cholinergic and glutamatergic hyperactivity and finally a predominantly glutamatergic phase after about 40 min (McDonough and Shih, 1997).

Exposure to nerve agent requires immediate medical treatment. For this purpose, a number of armed forces have based their therapy against nerve agent intoxication on an oxime (obidoxime (1,1′-(oxydimethylene)bis(4-formylpyridinium) dioxime), 2-PAM (2-[(hydroxyimino)methyl]-1-methylpyridinium chloride), HI-6 (1-[([4-(aminocarbonyl)pyridino]methoxy)methyl]-2-[(hydroxyimino)methyl]pyridinium)), an anticholinergic (atropine), and a $GABA_A$ (γ-amminobutyric acid A receptors) agent (diazepam, avizafone) combined with carbamate (pyridostigmine) pretreatment (Aas, 2003). However, such treatment regimens can reduce immediate lethality, but they do not attenuate the occurrence of nerve agent-induced seizure activity and concomitant convulsions, unless atropine is given early and at a high dose (McDonough and Shih, 1997). Such seizures rapidly progress to status epilepticus, a condition that is strongly associated with mortality and brain damage in experimental animals (Shih et al., 2003). In the search for novel strategies able to prevent or terminate nerve agent-evoked seizures, soman has been used in animal models because it takes a higher dose of anticonvulsants to stop seizures triggered by soman than other classical nerve agents (tabun, sarin, cyclosarin, VX). This finding suggests that drugs effective against soman will also be effective against other nerve agents (Shih and McDonough, 2000).

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Seizures that have lasted more than 40 min are gradually more difficult to terminate (Carpentier et al., 2001; Lallement et al., 1999). The refractory nature of sustained seizures represents a great challenge in treatment of nerve agent poisoned victims long time after exposure. The increased glutamatergic activity induced by soman causes excitotoxic damage and cell death (Munirathinam and Bahr, 2004), and the initial signs of injury are seen about 20 min after seizure onset (Lallement et al., 1994; McDonough et al., 1995). Seizure activity lasting 30 min or more (status epilepticus) has been shown to cause up-regulation of NMDA (N-methyl-D-aspartic acid) and AMPA (α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors along with internalization of $GABA_A$ receptors in hippocampal slices (Chen and Wasterlain, 2006; Wasterlain and Chen, 2008). This outcome means that $GABA_A$ receptors are inactivated, because they are no longer within reach of the neurotransmitter. Furthermore, NMDA and AMPA receptor subunits move to the synaptic membrane where they form additional excitatory receptors (Chen and Wasterlain, 2006; Wasterlain and Chen, 2008). These configurations may in part explain why glutamatergic antagonists and GABAergic agonists become gradually ineffective during the development of status epilepticus.

Given the implication of glutamatergic neurotransmission in epileptiform discharges, alternative targets for control of glutamatergic activity are of great interest. Through a large number of studies, metabotropic glutamate receptors have been shown to fulfill unique presynaptic and postsynaptic roles (Alexander and Godwin, 2006). In contrast to ionotropic glutamate receptors, which mediate fast synaptic transmission, metabotropic glutamate receptors often modulate on-going activity. When located postsynaptically, metabotropic glutamate receptors may modulate membrane properties by second messenger interactions, whereas presynaptic metabotropic glutamate receptors have been shown to control neurotransmitter release (Alexander and Godwin, 2006). These modulatory aspects appear to have attracted attention in experimental epilepsy. Group I metabotropic glutamate receptor antagonists and Group II metabotropic glutamate receptor agonists have been demonstrated to exert anticonvulsant efficacy, whereas Group III metabotropic glutamate receptor agonists show mixed responses in animal models of epilepsy (Alexander and Godwin, 2006).

We have previously examined the anticonvulsant potency of metabotropic glutamate receptor modulators microinfused into the perirhinal cortex of rats. The results show that the metabotropic glutamate receptor 5 antagonist MPEP hydrochloride (2-Methyl-6-(phenylethynyl)pyridine hydrochloride) and the metabotropic glutamate 2/3 receptor agonist DCG-IV ((2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine) cause full protection against seizures or increased latency to onset of seizures, whereas the metabotropic glutamate receptor 1 antagonist LY367385 ((S)-(+)-α-Amino-4-carboxy-2-methylbenzeneacetic acid) does not produce anticonvulsant efficacy in response to systemically administered soman (Myhrer et al., 2010). The present study was designed to investigate the anticonvulsant capacities of MPEP and DCG-IV during systemic administration. However, in contrast to microinfusion studies in which anticonvulsant action of a single drug can be measured, combinations of drugs are necessary for achieving antiseizure efficacy when systemic administration is used (Myhrer et al., 2011). Both MPEP and DCG-IV pass the blood-brain barrier (Jesse et al., 2008; Tomita et al., 2000).

Procyclidine has been shown to be the most potent anticonvulsant tested in our microinfusion studies, and its potency can be further enhanced when combined with the antiepileptic drug levetiracetam (Myhrer et al., 2011). Inclusion of the oxime HI-6 serves as an important factor in optimizing the antidotal efficacy of procyclidine and levetiracetam (Myhrer et al., in press). The purpose of the present study was to examine antidotal effects of MPEP or DCG-IV combined with either HI-6 and procyclidine or HI-6 and levetiracetam relative to the combination of HI-6, procyclidine, and levetiracetam 20 min after onset of seizures elicited by soman in rats pretreated with pyridostigmine. Treatment later than 20 min after seizure onset requires pretreatment with HI-6 in order to have a reasonable number of surviving rats, but this oxime may mask genuine anticonvulsant effects because of several pharmacological actions (Myhrer et al., 2011). The most potent combinations were also tested as prophylactic therapies and were for that purpose given 20 min before soman poisoning. Because MPEP has been reported to protect acetylcholinesterase activity against inhibition caused by the cholinergic agonist pilocarpine (Jesse et al., 2008), it was investigated whether MPEP could prevent inhibition of acetylcholinesterase activity by soman.

2. Materials and methods

2.1. Animals

Male Wistar rats from a commercial supplier (Taconic Breeding Laboratories, Denmark) weighing 300–330 g served as subjects. The experiments were approved by the National Animal Research Authority. The animals were housed individually and had free access to commercial rat pellets and water. The rats were handled individually 3 days preoperatively and 3 days postoperatively, being allowed to explore a table top ($80 \times 60\ cm^2$) for 3 min a day. The climatized vivarium (21 °C) was illuminated from 0700 to 1900 h.

2.2. Surgery

The rats were anesthetized ip with diazepam (4.5 mg/kg) and fentanyl fluanisone (2 mg/kg). Of 2 stainless screws, one was lowered 1 mm into the parietal cortex (1 mm behind bregma, 3 mm lateral to midline), and the contralateral one served as ground. The screws were fixed with dental cement (Durelon; ESPE, Seefeldt, Germany). The rats were given a recovery period of 7 days.

2.3. Drugs and nerve agent

Some of the drug doses chosen were derived from previous studies of anticonvulsant effects against soman-evoked seizures in rats; HI-6 dimethanesulphonate 125 mg/kg, procyclidine hydrochloride 6 or 20 mg/kg, levetiracetam (Keppra®) 50 mg/kg, pyridostigmine bromide 0.1 mg/kg (Myhrer et al. 2011, in press). MPEP doses (15, 30, or 60 mg/kg) were derived from a study in which seizures were induced by electrical stimulation (Lojková and Mareš, 2005). The dose of 4 mg/kg DCG-IV was established from our own pilot experimentation of anticonvulsant effect against soman-evoked seizures. The drugs were dissolved in 0.9% saline, except MPEP that was dissolved in 10% dimethyl sulfoxide in distilled water (33% 2-hydroxy propyl-β-cyclodextrin) (Ali, 2001). All drugs were administered intraperitoneally. The soman dose was $1.3 \times LD_{50}$ (100 μg/kg) resulting in convulsions and death in all rats of our strain (Sterri et al., 1980). Soman was given subcutaneously. Procyclidine and pyridostigmine were purchased from Sigma (St Louis, Missouri, USA), and MPEP and DCG-IV were purchased from Tocris Cookson Ltd (Bristol, United Kingdom). HI-6 dimethanesulphonate was a gift from Defence Research and Development (Suffield, Medicine Hat, Canada).