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Enhanced efficacy of anticonvulsants when combined with levetiracetam in soman-exposed rats

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ABSTRACT

Results from studies based on microinfusions into seizure controlling brain sites (area tempestas, medial septum, perirhinal cortex, posterior piriform cortex) have shown that procyclidine, muscimol, caramiphen, and NBQX, but not ketamine, exert anticonvulsant effects against soman-induced seizures. The purpose of the present study was to examine whether levetiracetam (Keppra®) may enhance the anticonvulsant potency of the above drugs to become optimally effective when used systemically. Levetiracetam has a unique profile in preclinical models of epilepsy and has been shown to increase the potency of other antiepileptic drugs. The rats were pretreated with pyridostigmine (0.1 mg/kg) to enhance survival and received anticonvulsants 20 min after onset of seizures evoked by soman ($1.15 \times LD_{50}$). The results showed that no single drug was able to terminate seizure activity. However, when levetiracetam (LEV; 50 mg/kg) was combined with either procyclidine (PCD; 10 mg/kg) or caramiphen (CMP; 10 mg/kg) complete cessation of seizures was achieved, but the nicotinic antagonist mecamylamine was needed to induce full motor rest in some rats. In a subsequent experiment, rats were pretreated with HI-6 (125 mg/kg) to enhance survival and treatment started 40 min following seizure onset of a soman dose of $1.6 \times LD_{50}$. LEV (50 mg/kg) combined with either PCD (20 mg/kg) or CMP (20 mg/kg) terminated seizure activity, but the survival rate was considerably higher for LEV + PCD than LEV + CMP. Both therapies could also save the lives of rats that were about to die 5–10 min after seizure onset. Thus, the combination of LEV and PCD or CMP may make up a model of a future autoinjector being effective regardless of the time of application.

1. Introduction

Organophosphorus nerve agents are lethal chemical warfare means that may be encountered during military combats, terrorist use, or during chemical disarmament. Nerve agents act by irreversibly inhibiting acetylcholinesterase, the enzyme that hydrolyzes acetylcholine. Accumulation of acetylcholine results in excessive stimulation of muscarinic and nicotinic receptors. The signs of poisoning are seen as increased salivation, respiratory distress, tremor, seizures/convulsions, coma, and death (Taylor, 2001). Increased cholinergic activity in the brain is probably related to the initial phase of seizures, whereas sustained seizures are probably associated with increased glutamatergic activity leading to neuronal damage (McDonough and Shih, 1997).

Medical management of nerve agent poisoning is based on pretreatment with a carbamate cholinesterase inhibitor (pyridostigmine) to shield a fraction of the cholinesterase from irreversible inhibition by the nerve agent. Treatment after exposure to nerve agent is based on a cholinergic antagonist (atropine sulfate) along with an oxime (obidoxime, 2-PAM, HI-6) to reactivate any unaged inhibited enzyme (Aas, 2003). Such treatment regimen can increase the survival rate significantly, but it does not effectively reduce nerve agent-induced seizure activity resulting in brain injury. For this reason, efforts in search for effective countermeasures have aimed at drugs exerting cholinergic and glutamatergic antagonism along with GABAergic agonism (McDonough and Shih, 1997). However, determination of critical receptor subtypes would provide clues for the designing of more specific anticonvulsive therapeutic strategies as it has been made in epilepsy research. Within the rat brain, there are control mechanisms capable of attenuating all aspects of convulsive activity. Several target areas have been identified, and it is assumed that the ability of a systemically administered drug to confer seizure protection depends on the drug's relative impact on the defined action sites (Gale, 1988).

A series of recent lesion and microinfusion studies have been devoted to the identification of control sites for soman-induced seizure activity and specification of critical receptors for pharma-

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cological management. It has been shown that lesion of the area tempestas, medial septum, perirhinal cortex, or posterior piriform cortex produces anticonvulsant effects (prevention of convulsions or delayed onset of convulsions) in rats exposed to soman, whereas damage to nucleus accumbens, nucleus basalis magnocellularis, amygdala, hippocampus, or entorhinal cortex does not cause anticonvulsant impact (Myhrer et al., 2007, 2008a). These results are in compliance with findings that seizures can be generated in area tempestas, medial septum, perirhinal cortex, and posterior piriform cortex by means of nerve agents, chemoconvulsants, or kindling (Myhrer, 2010). Results from microinfusion studies show that anticonvulsant efficacy is obtained by $GABA_A$ modulators (muscimol, ethanol, propofol) or cholinergic antagonists (M1–M5) (atropine, scopolamine, caramiphen, procyclidine) in area tempestas (Myhrer et al., 2006a, 2008b), cholinergic antagonists (M1–M5) (atropine, scopolamine, procyclidine) in medial septum (Myhrer et al., 2009), combined glutamatergic (NMDA) and cholinergic antagonist (M1–M4) (procyclidine), AMPA antagonist (NBQX), or modulators of metabotropic glutamate receptors (mGluR5, mGluR2/3) (MPEP, DCG-IV) in the perirhinal cortex (Myhrer et al., 2010a,b), and cholinergic antagonist (M1–M5) (scopolamine) or $GABA_A$ agonist (muscimol) in the posterior piriform cortex (Myhrer et al., 2010a). Calculation of impact factors for the most potent drugs (percentage of positive effects in the seizure controlling sites) showed that scopolamine and procyclidine were ranking highest (75) followed by muscimol (50), NBQX (33), and caramiphen (33), whereas the impact factor of ketamine was 0 (Myhrer, 2010). When the percentage of nonconvulsing rats from both lesion and microinfusion studies is used as guidance for selecting the most influential seizure controlling brain sites, the area tempestas and perirhinal cortex stand out as the most prominent ones. Procyclidine exerts anticonvulsant efficacy in both areas (high impact), whereas scopolamine, NBQX, caramiphen, and muscimol yield anticonvulsant effects in 1 of the areas only (low impact) (Myhrer, 2010).

The high anticonvulsant potency of scopolamine will have limited use as a post-exposure agent, because the comparatively narrow cholinergic window of the 3-phase model (about 5 min after seizure onset) makes the efficacy of anticholinergics gradually weaker with elapse of time since seizure onset (McDonough and Shih, 1997). On the other hand, procyclidine (impact factor 75) has proved very useful as a post-exposure means when combined with either muscimol, ethanol, or propofol. However, these GABAergic modulators can depress respiratory function, and about 17% of the rats died within 24 h with each treatment regimen (Myhrer et al., 2006b). To avoid adverse effects on the brainstem, enhancement of procyclidine's anticonvulsant impact on the seizure controlling sites of the forebrain would make up a novel and interesting approach. Levetiracetam with a unique profile in preclinical models of epilepsy has been shown to increase the potency of other antiepileptic drugs up to 19-fold (Kaminski et al., 2009).

The purpose of the present study was to make a comparative assessment of anticonvulsant effects of levetiracetam, procyclidine, muscimol, caramiphen, NBQX, and ketamine or each drug in combination with levetiracetam 20 min after onset of soman-evoked seizures (experiment 1). In experiment 2, levetiracetam was combined with either procyclidine or caramiphen and the treatment started 40 min following seizure onset. In attempt to prolong survival, the rats were pretreated with pyridostigmine in experiment 1 and with HI-6 in experiment 2. A single dose of each anticonvulsant previously shown to cause optimal efficacy was applied. One exception was the use of 2 doses with procyclidine and caramiphen in combination with levetiracetam in experiment 1 to examine impact of nicotinic antagonism on motor dysfunctions after seizure termination. Additionally, mecamylamine was given as adjunct in both experiments. It was also of interest to investigate whether the most potent combination(s) with levetiracetam may prevent onset of seizure activity (experiment 2).

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 cm × 60 cm) 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, and the contralateral one served as ground. The screws were fixed with dental cement (Durelon; ESPE, Seefeldt, Germany). The electrodes were connected with the polygraph (Grass Model 79E) with alligator clips and leads. The use of a swivel allowed the rats to move freely. The rats were given a recovery period of 7 days.

2.3. Drugs

The drug doses chosen were derived from previous studies of anticonvulsant effects against soman-evoked seizures in rats; procyclidine hydrochloride 10 mg/kg, caramiphen edisylate 10 mg/kg, muscimol hydrobromide 20 mg/kg, NBQX 40 mg/kg, ketamine hydrochloride 50 mg/kg (Lallement et al., 1994; Myhrer et al., 2006b; Raveh et al., 2003; Shih et al., 1999) or antiepileptic effects in preclinical epilepsy models; levetiracetam 50 mg/kg (Kaminski et al., 2009). HI-6 dimethanesulphonate (125 mg/kg) was used to reduce death, since this oxime has been demonstrated to cause a survival rate of 60% 24 h after a soman dose of 1.6 × LD_{50} (Shih et al., 1991). The carbamate pyridostigmine bromide (0.026 mg/kg) has also been used to prolong survival among rats (Scremin et al., 1997). Results from our pilot experiment showed that when the soman dose is 1.15 × LD_{50}, the survival rate 20 min following seizure onset is 50%. This percentage is increased to 75% when pyridostigmine (0.1 mg/kg) is administered 20 min before soman exposure. Mecamylamine (10 mg/kg) (Shih et al., 1991) was given to rats that displayed nicotinic motor dysfunctions after termination of seizures. The soman doses were either 1.15 × LD_{50} (92 μg/kg) resulting in convulsions and death in 85% of the rats or 1.6 × LD_{50} (128 μg/kg) resulting in convulsions and death in all rats of our strain (Sterri et al., 1980). When using a prophylactic regimen, the soman dose was 1.3 × LD_{50} (100 μg/kg) to ensure that all rats would convulse without appropriate pretreatment. Soman was given sc. All drugs were purchased from Sigma (St Louis, MO, USA), except HI-6 that was a gift from Defence Research and Development (Canada). The drugs were dissolved in 0.9% saline, and control rats in experiment 2 received 0.3 ml of saline (0.9%) × 2 as treatment. The drugs were administered ip.

2.4. Experimental design

For overview, see Fig. 1. Experiment 1. (A) Pyridostigmine given 20 min before soman (1.15 × LD_{50}). Testing anticonvulsant effect of each drug (levetiracetam, procyclidine, caramiphen, muscimol,