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Pharmacological therapies against soman-induced seizures in rats 30 min following onset and anticonvulsant impact

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Abstract

Systemic administration does not allow a clear differentiation between the anticonvulsant properties of $GABA_A$ (γ-aminobutyric acid) modulators. For this reason, various $GABA_A$ modulators have previously been micro-infused into seizure controlling substrates (area tempestas, substantia nigra) in the rat brain as a screening method for potential systemic administration. The purpose of the present study was to examine the anticonvulsant impact of the GABAergic modulators muscimol, ethanol, and propofol (screened by micro-infusions) when each drug was combined with procyclidine and administered systemically. The results showed that all 3 combinations could effectively terminate soman-induced (100 μg/kg s.c.) seizures when administered 30–35 min after onset. Procyclidine and propofol were considered as the most relevant double regimen to replace a previous triple regimen (procyclidine, diazepam, pentobarbital) against soman-induced seizures. Additionally, it was shown that unilateral implantation of hippocampal electrodes resulted in increased resistance to aphagia/adipsia and neuropathology, but not to lethality following soman. Efficient pharmacological treatment of soman-induced seizures at an early stage (<20 min) is crucial to avoid neuropathology and cognitive deficits.

Keywords: Soman; Seizure; Rocyclidine; Muscimol; Ethanol; Propofol; Aphagia/adipsia; Resistance; Electrode placement

1. Introduction

The organophosphorus nerve agent soman is a highly potent inhibitor of the enzyme acetylcholinesterase. Such inhibition results in a rapid accumulation of acetylcholine and over-stimulation of muscarinic and nicotinic receptors. The toxic signs include hyper-secretion, respiratory distress, tremor, seizures/convulsions, coma, and death (Taylor, 2001). The elevated cholinergic activity in the brain likely induces the initial phase of seizures (Lallement et al., 1992; McDonough and Shih, 1997), and sustained seizures are probably associated with increased glutamatergic activity leading to excitotoxic lesions predominantly in the piriform cortex, entorhinal cortex, amygdala, and hippocampus (Carpentier et al., 1991; McDonough et al., 1995; McDonough and Shih, 1997).

Soman-induced seizures that have lasted more than 10 min seem to be difficult to terminate unless the countermeasures exert cholinergic and glutamatergic antagonism as well as GABAergic (γ-aminobutyric acid) agonism (McDonough and Shih, 1997). It will take at least 30 min for emergency personnel to access individuals unprepared for exposure to nerve agent. Furthermore, even soldiers properly provided with protective mask, gloves and clothes may need medical help, because bad training, bad discipline or bad luck can lead to intoxication of nerve agent (cf. Lallement et al., 1999). Thus, there has been an urgent need for strategies capable of terminating soman-induced seizures 30–40 min following onset. In a recent study, it was demonstrated that a triple regimen consisting of procyclidine (6 g/kg), diazepam (10 mg/kg), and pentobarbital (30 mg/kg) can effectively terminate soman-induced seizures in rats when administered intraperitoneally 5 min apart 30–40 min following onset (Myhrer et al., 2003). The notion of this triple regimen was derived from the finding that combined use of the $GABA_A$ modulator, diazepam and pentobarbital, can terminate seizures induced by kainic acid or lithium/pilocarpine in rats 1 h after onset (Du et al., 1995), and that procyclidine exerts antagonism at both muscarinic and nicotinic receptors and additionally has antagonistic effects at NMDA (*N*-Methyl-D-Aspartate) receptors (Kim et al., 2002).

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In both military and civilian contexts, it would appear somewhat inconvenient for medical personnel to administer 3 injections of antidotes to achieve termination of seizure activity. It would be more expedient to replace diazepam and pentobarbital by a single $GABA_A$ modulator assuring corresponding anticonvulsant impact. However, systemic administration of $GABA_A$ modulators does not differentiate well between their anticonvulsant properties (Shih et al., 1999). In a recent study based on micro-infusion of GABAergic modulators into seizure controlling areas in the rat brain (area tempestas, substantia nigra), it was found that muscimol, ethanol and propofol possessed the best anticonvulsant potencies against soman seizures among the agents examined (Myhrer et al., 2006). Muscimol acts at the $GABA_A$ binding site and is classified as a direct agonist. While propofol and ethanol can produce agonist-like effects, they are considered as receptor site modulators and not direct $GABA_A$ agonists. Collectively, muscimol, propofol, and ethanol are termed GABAergic modulators in this report. The purpose of the present study was to examine whether systemic administration of procyclidine in combination with either muscimol, ethanol, or propofol can effectively terminate soman-induced seizures 30–35 min after onset. An additional objective of this study was to investigate whether the implantation of hippocampal electrodes or the surgery alone might influence the resistance against lethality and aphagia/adipsia in soman intoxicated rats. A comparison of the results between 2 previous studies showed that the mortality rate was higher among rats without electrodes (60%) than among those with hippocampal electrodes (17%). Furthermore, 71% of rats without electrodes displayed aphagia, whereas only 10% of rats with hippocampal electrodes suffered from aphagia (Myhrer et al., 2003, 2005). In the present study, the rats in each pharmacological treatment group were either implanted with hippocampal or cortical electrodes or remained unoperated as controls.

2. Materials and methods

2.1. Subjects

Forty-five male Wistar rats from a commercial supplier (Harlan, The Netherlands) weighing 300–350 g served as subjects. All rats included in this study convulsed in response to soman. 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 × 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 anaesthetized i.p. 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. Bipolar electrodes were implanted stereotaxically in the dorsal hippocampal region of the left hemisphere. The electrodes were made from insulated silver thread of 0.3 mm in diameter (Johnson Matthey Metals Ltd., USA) each soldered to a male golden pin component (220-PO2100 Bunker Ramo, Amphenol North America, USA). The electrodes were twisted and cut so that one tip was 0.7 mm shorter than the other. Only the tips were bared of insulation (about 0.3 mm), and they were spaced 1.5 mm from one another. The electrode pair was placed with one tip in the CA1 pyramidal layer and the other one in the granular layer of the fascia dentata. Such placement of electrodes permits the recording of clear large amplitude theta activity either bipolarly or unipolarly (Bland, 1986). The central implantation coordinates for the set of 2 twisted electrodes were 3.2 mm behind bregma, 2.0 mm lateral to the midline, and 3.0 mm depth from skull level. The electrodes were inserted vertically with the top of the skull in horizontal position. The electrode pair was longitudinally oriented with the deepest electrode in posterior position and 0.7 mm lateral to the anterior one. More specifically, the CA1 electrode was inserted 1.5 mm lateral to the midline in a depth of 2.3 mm, corresponding to Level 3.6 in the stereotaxic atlas of Pellegrino and Cushman (1967). The fascia dentata electrode was inserted 3 mm lateral to the midline in a depth of 3 mm, corresponding to Level 2.8 of Pellegrino and Cushman (1967). The electrodes were fixed with steel screws and dental cement. The rats were grounded to the recording polygraph (Grass Model 7P5A) from one of the screws in the skull. The leads were connected to a five-channel swivel that allowed the rats to move freely. The cortical screw electrodes were connected to the polygraph with alligator clips and leads. The rats were given a recovery period of 7 days.

2.3. Drugs

Procyclidine hydrochloride (Sigma Chemical Co, St Louis, MO, USA) was dissolved in 0.9% saline and injected in a dose of 10 mg/kg. Muscimol hydrobromide (Sigma) was dissolved in 0.9% saline and used in a dose of 20 mg/kg. Ethanol was diluted to 25% and injected in a dose of 3 mg/kg. Propofol (Diprivan®) was used in a dose of 50 mg/kg. The doses applied were based on results from previous pilot experiments and were administered separately as i.p. injections. Soman was administered 100 μg/kg s.c. in an injection volume of 100 μg/ml in saline (0.9%). Anticonvulsant treatment started 30 min after onset of soman-induced seizures with injections spaced 5 min apart to avoid respiratory depression.

2.4. Histology

The rats were sacrificed 48 h following exposure to soman. They were anesthetized, perfused intracardially with 10% formalin, and the brains post-fixed in 10% formalin for at least 24 h. The brains were dehydrated and embedded in paraffin (Schmued et al., 1997). The sections were cut 5 μm thick and dried in an incubator (37 °C) for 12 h before they were stained with hematoxylin and eosin (HE) or Fluoro-Jade B (Schmued and Hopkins, 2000). Because Fluoro-Jade has been considered