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Behavioral side effects in rats treated with acetylcholinesterase inhibitors suggested used as prophylactics against nerve agents

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ABSTRACT

Acetylcholinesterase inhibitors in combination with an anticholinergic, particularly anticholinergics with antiglutamatergic properties, can effectively protect against nerve agent-induced seizures and lethality. The objective of the present study was to examine potential behavioral side effects of the anticholinesterases physostigmine (0.1 mg/kg), galantamine (3 mg/kg), huperzine (0.5 mg/kg), and donepezil (2.5 mg/kg) alone or each drug in combination with anticholinergic procyclidine (3 mg/kg). The results showed that rats injected intraperitoneally with galantamine displayed a mild cognitive deficit in terms of reduced preference for novelty that was similarly found among animals treated with procyclidine combined with either galantamine or donepezil. Locomotor activity and rearing were radically depressed in all groups treated with anticholinesterases as well as in combination with procyclidine. Reductions in activity were most prominent for rats injected with galantamine alone. Equalizing effects of cholinesterase inhibitors and anticholinergics were absent in the present context. Findings from previous studies that both systemic and local (amygdala) application of physostigmine cause increased fear-motivated freezing response in rats, may explain the marked reductions in activity among the present rats. In view of these findings, use of anticholinesterases (crossing the blood-brain barrier) as prophylactics against nerve agents must be carefully examined to avoid severe side effects.

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. Increased cholinergic activity in the brain is probably related to the initial phase of seizures (McDonough and Shih, 1997; Lallement et al., 1992), whereas sustained seizures are probably associated with increased glutamatergic activity leading to neuronal damage predominantly in the hippocampus, amygdala, piriform cortex, and entorhinal cortex (McDonough and Shih, 1997; Carpentier et al., 1991).

In order to prevent lethality by soman [-(1,2,2-trimethyl-propyl) methyl-phosphonofluoridate] it is important to shield temporarily a portion of the acetylcholinesterase from irreversible inhibition followed by the therapeutic treatment with an anticholinergic drug. To meet these requirements, a number of military forces have based their medical therapy on pyridostigmine pretreatment to prevent acetylcholine inhibition by nerve agents followed by the immediate therapeutic treatment with atropine sulfate and an oxime administered by one or more autoinjectors. These drugs are intended to inhibit muscarinic receptors and to reactivate any “unaged” enzyme, respectively, following exposure to nerve agent (Aas, 2003). However, since pyridostigmine does not readily cross the blood-brain barrier, physostigmine that readily enters the brain, has been suggested as a possible replacement. In studies of guinea pigs and rats, evidence has been presented that effective prevention of soman-induced lethality can be assured by physostigmine in combination with scopolamine or procyclidine (Kim et al., 2002; Choi et al., 2004; Myhrer et al., 2004b, Philippens et al., 2000; Wetherell et al., 2002). Pyridostigmine combined with caramiphen or benactyzine and trihexyphenidyl or with biperiden have also been reported to provide efficacious pretreatment in soman-poisoned rats (Bajgar, 2004; Kassa et al., 2003; Raveh et al., 2003).

The half-life of physostigmine is relatively short. For this reason, the Alzheimer drugs donepezil, galantamine, and huperzine with relatively long half-lives have been suggested as possible alternative prophylactic cholinesterase inhibitors against nerve agent intoxication (Aas, 2003). Donepezil is a partial reversible centrally acting and highly selective inhibitor of the acetylcholinesterase (Sugimoto et al., 2002). Galantamine is another drug approved for treatment of mild to moderate Alzheimer's disease. The drug is a reversible acetylcholinesterase inhibitor that crosses the blood-brain barrier (Corey-Bloom, 2003).

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Huperzine is a slow, reversible inhibitor of the acetylcholinesterase at both peripheral and central levels (Ashani et al., 1992). This drug is used for treatment of Alzheimer's disease in China (Wang et al., 2000).

Acetylcholinesterase inhibitors are not very efficacious prophylactics against nerve agent poisoning if they are administered alone. Their efficacy is considerably enhanced when combined with an anticholinergic agent like atropine. However, the anticonvulsant impact may be even further improved if acetylcholinesterase inhibitors are administered along with an antiparkinson drug. This group of agents possesses potent anticonvulsant properties against nerve agents, because the drugs exert both cholinergic and glutamatergic antagonism in mice and rats (Gao et al., 1998; McDonough and Shih, 1995; Raveh et al., 2002). The antiglutamatergic effect appears particularly relevant, since glutamatergic pathways have been suggested to be intimately involved in the early stages of soman-induced seizures (Weissman and Raveh, 2008). For the present purpose, procyclidine was chosen. This drug combined with either physostigmine or donepezil can effectively prevent soman-generated seizures and lethality in rats (Kim et al., 2002; Haug et al., 2007; Myhrer et al., 2004b). Procyclidine does not seem to have been examined in combination with galantamine or huperzine in previous nerve agent studies.

Because seizures are associated with both lethality and brain damage (Shih et al., 2003), it is very important to prevent the onset of seizures or terminate seizures within 20 min after onset to avoid neuropathology (Lallement et al., 1994; McDonough et al., 1995). However, a crucial matter is whether the doses of prophylactics required for protection of military personnel against nerve agent-induced damage will impair cognitive functions. The purpose of the present study was to make a comparative assessment of potential behavioral effects of procyclidine, donepezil, galantamine, huperzine, and physostigmine (Experiment 1) or each acetylcholinesterase inhibitor in combination with procyclidine (Experiment 2). The doses of drugs chosen have previously been shown to have anticonvulsant effects against soman-induced seizures. The behavioral task employed was a novelty test that has proven particularly sensitive in revealing cognitive dysfunctions following selective disruptions of entorhinal projections (Myhrer, 1988, 1989). Exploration of a discrete novel object is one form of inquisitive activity frequently seen among rats. This activity appears as a strong preference for novelty, the recognition of which is probably based on polymodal sensory information (Berlyne, 1960). The rats were tested in a modified version of the novelty test of Berlyne (1950) consisting of three different sets of stimuli; visual/tactile, olfactory, or visual only (Myhrer, 1988).

2. Materials and methods

2.1. Animals

2.1.1. Experiment 1

Forty-eight male Wistar albino rats from a commercial supplier (Taconic Breeding Laboratories, Denmark) weighing 280–310 g when the experiment started, served as subjects. The rats were randomly assigned to one of 6 groups (8 rats in each) and their group assignment was unknown during testing. The various groups received i.p. injection of either saline, procyclidine, donepezil, galantamine, huperzine, or physostigmine. The rats were housed individually and had free access to commercial rat pellets and water. With the novelty test used, reliable results are dependent on emotionally stable animals. For this reason, the rats were handled individually 7–10 days, being allowed to explore a table top (80×60 cm) for 3 min a day. The climatized (21 °C) vivarium was illuminated from 0700 to 1900 h.

2.1.2. Experiment 2

Forty male Wistar rats (280–310 g) from the same supplier served as subjects. The animals were randomly assigned to one of 5 groups with 8 rats in each. The various groups received i.p. injection of saline or procyclidine combined with either donepezil, galantamine, huperzine, or physostigmine. The rats were treated as described for Experiment 1.

The experiments were approved by the National Animal Research Authority. A minimal number of animals were used, and all efforts were made to avoid animal suffering according to the European Communities Council Directive of 1986 (86/609/EEC).

2.2. Drug administration

Physostigmine salicylate (Sigma-Aldrich) was dissolved in physiological saline (0.9%) and administered in standardized dose of 0.1 mg/kg (Myhrer et al., 2004b). Donepezil hydrochloride was obtained as 5 mg tablets (Aricept®, Pfizer). The tablets were crushed, suspended in saline (2 mg/ml) and given in a dose of 2.5 mg/kg (Haug et al., 2007). Although donepezil is water soluble, the suspension was injected instead of just the aqueous extract to ensure a homogenous administration. Huperzine A (Sigma-Aldrich) was dissolved in saline and injected in a dose of 0.5 mg/kg (Tonduli et al., 2001). Galantamine hydrobromide (Sigma-Aldrich) was dissolved in saline and given in a dose of 3 mg/kg that attenuates cognitive impairment induced by medial septal lesions in rats (Mulder et al., 2005). Galantamine does not seem to have been used against soman in rats. In guinea pigs, however, anticonvulsant doses of 5 or 8 mg/kg of galantamine have been used against soman (Albuquerque et al., 2006). Thus, the dose of 3 mg/kg for rats appears rather conservative. Procyclidine hydrochloride (Sigma-Aldich) was dissolved in saline and administered in a dose of 3 mg/kg (Myhrer et al., 2004a). The drugs were given 20 min before each test session (one session a day for 3 days) with a total testing period of 20 min. One exception was huperzine that was given 40 min before each test session, because stable acetylcholinesterase inhibition is obtained after 40 min in rats (Tonduli et al., 2001). When procyclidine was combined with anticholinesterases (Experiment 2), the injections were given in rapid succession (procyclidine first, also in combination with huperzine). Physiological saline was injected i.p. in a volume of 0.3 ml. Prophylactics are usually given 20 or 30 min before exposure to nerve agent (Myhrer et al., 2008).

2.3. Apparatus

Behavioral testing was carried out in a Plexiglas cage (54×33×20 cm) previously described (Myhrer, 1988). In brief, the floor was divided in 18 equal squares (9×11 cm). Three identical aluminum cubes (5×5×5 cm) were evenly distributed in the cage in fixed positions (the neutral objects). Three other cubes made up the novel objects. One object only differed from the neutral ones in that its top was uneven with tracks (2 mm) in it making up a square pattern (visual/tactile stimuli). Since the rats could perceive the tracks or the squares (16 squares measuring 1.1×1.1 cm) by bodily contact, both tactile and visual sensory modalities might be used. One was identical with the neutral ones, and a spot of cheese (dia. 1.5 cm) was smeared on the side facing the experimenter (olfactory stimulus). So-called Norwegian white cheese (Norvegia) that hardly smells at all to humans was used. In the test cage, it was not possible to detect the cheese visually. One was smaller than the neutrals, (4.5×4.5×4.5 cm) and two sides were slightly uneven (visual stimulus). All objects were painted light gray. The sound attenuated testing room was provided with a fan producing white noise (52 dB).

2.4. Procedure

The same procedure was followed for both Experiments 1 and 2. During adaptation, the rats were allowed to explore individually the empty apparatus for 20 min. On the next day, the rats were given the test drugs before they were run in Session I. In Phase 1, the animals were tested for 5 min in the test cage with three neutral objects present. Then the rats spent 10 min in the home cage. In Phase 2, the rats were tested again for 5 min, and the neutral object in the middle