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Antiparkinson drugs used as prophylactics for nerve agents: Studies of cognitive side effects in rats

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ABSTRACT

Antiparkinson agents possess excellent anticonvulsant properties against nerve agent-induced seizures by exerting both cholinergic and glutamatergic antagonisms. It is important, however, that drugs used as prophylactics not by themselves cause impairment of cognitive capability. The purpose of the present study was to make a comparative assessment of potential cognitive effects of benactyzine (0.3 mg/kg), biperiden (0.11 mg/kg), caramiphen (10 mg/kg), procyclidine (3 mg/kg), and trihexyphenidyl (0.12 mg/kg) separately and each in combination with physostigmine (0.1 mg/kg). The results showed that benactyzine, caramiphen, and trihexyphenidyl reduced rats' innate preference for novelty, whereas biperiden and procyclidine did not. When benactyzine, caramiphen, and trihexyphenidyl were combined with physostigmine the cognitive impairment disappeared. This counteracting effect, however, caused changes in locomotor and rearing activities not seen by each drug alone. Acetylcholinesterase inhibitors and anticholinergics used as prophylactics can offset each other, but exceptions are observed in a previous study when a very potent anticholinergic (scopolamine) or a high dose of procyclidine still results in cognitive deficits in spite of coadministration with physostigmine. Among the present drugs tested, procyclidine appears to be a robust anticonvulsant with few cognitive side effects.

1. Introduction

Nerve agents consist of a group of highly toxic organophosphates that acts 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 hypersecretion, 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). Because it takes a higher dose of anticonvulsants to terminate seizures induced by soman (pinacolyl methylphosphonofluoridate) than by other classical nerve agents (Shih and McDonough, 2000), soman is considered to be the most relevant agent to be used in animal models to evaluate the anticonvulsant potency of pharmacological agents.

Prophylactic treatment against nerve agents can be obtained by shielding temporarily a fraction of the acetylcholinesterase from irreversible inhibition together with the therapeutic treatment with an anticholinergic drug. For this purpose, 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 an autoinjector. These drugs are meant 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 ensured 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 has also been reported to provide efficacious pretreatment in soman-poisoned rats (Bajgar, 2004; Kassa et al., 2003; Raveh et al., 2003).

The group of antiparkinson drugs including benactyzine, biperiden, caramiphen, procyclidine, and trihexyphenidyl (Gao et al., 1998; Vargas et al., 1998) possesses potent anticonvulsant properties against nerve agent-induced seizures, since these drugs exert both cholinergic and glutamatergic antagonisms in mice and rats (Gao et al., 1998; McDonough and Shih, 1995; Raveh et al., 2002). Antiparkinson agents are therefore well suited as anticonvulsants against soman-evoked seizures. Because seizures are associated with both lethality and brain damage (Shih et al., 2003), it is very important to prevent the onset of

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seizures or terminate seizures within 20 min after onset to avoid neuropathology (Lallement et al., 1994; McDonough et al., 1995). A fixed dose of physostigmine (0.1 mg/kg) combined with various doses of procyclidine (1–6 mg/kg) can effectively protect against soman intoxication (1.3–2.0×LD_{50}) in a dose-related manner (Myhrer et al., 2004b). However, the higher the dose of procyclidine used, the more pronounced the cognitive side effects in rats (Myhrer et al., 2004a). Very low doses of physostigmine (0.015 and 0.03 mg/kg) in rats have been shown to enhance memory in a passive avoidance task (Santucci et al., 1989). However, the application of acetylcholine receptor antagonists will result in the opposite effect. Both atropine and scopolamine have convincingly been demonstrated to impair rats' performance in Morris water maze and spontaneous alternation (Myhrer, 2003). Furthermore, glutamatergic antagonists (MK-801, ketamine) cause performance deficits in the latter behavioral tasks as well as in radial maze and passive avoidance (Myhrer, 2003). Hence, a crucial matter is whether the doses of prophylactics required for protection against nerve agent-induced damage will impair cognitive functions. The purpose of the present study was to make a comparative assessment of potential cognitive effects of benactyzine, biperiden, caramiphen, procyclidine, and trihexyphenidyl (Experiment 1) or each drug combined with physostigmine (Experiment 2). 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. Methods

2.1. Animals

2.1.1. Experiment1

Forty-eight male Wistar rats from a commercial supplier (Møllegaard 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, benactyzine, biperiden, caramiphen, procyclidine, or trihexyphenidyl. 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-eight male rats (280–310 g) served as subjects. The animals were randomly assigned to the following treatment groups with 8 rats in each: i.p. treatment with saline (×2), physostigmine combined with either benactyzine, biperiden, caramiphen, procyclidine, or trihexyphenidyl. 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, benactyzine, caramiphen edisylate, procyclidine hydrochloride, and trihexyphenidyl hydrochloride were purchased from Sigma (St. Louis. MO, USA), and biperiden lact. was purchased from Abbot (Solna, Sweden). All drugs were dissolved in 0.9% physiological saline and administered i.p. in the following doses: physostigmine 0.1 mg/kg, benactyzine 0.3 mg/kg, biperiden 0.11 mg/kg, caramiphen 10 mg/kg, procyclidine 3 mg/kg, and trihexyphenidyl 0.12 mg/kg. These doses have previously been reported to assure anticonvulsant effects against soman when administered prophylactically 20 or 30 min before the nerve agent (Capacio and Shih, 1991; Kim et al., 2002; Myhrer et al., 2004b; Raveh et al., 2002; Shih et al., 1991). Furthermore, the selected dose of physostigmine does not by itself significantly affect the performance on the test used in this study (Myhrer et al., 2004b). The drugs were given 20 min before each test session (1 session a day for 3 days) with testing time of 20 min. When physostigmine was combined with antiparkinson drugs (Experiment 2), the injections were given in rapid succession (physostigmine first). Physiological saline was injected i.p. in a volume of 0.3 ml.

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

During adaptation, the rats were allowed to explore individually the empty apparatus for 20 min. On the next day, the rats were run in Session I. In Phase 1, the animals were tested for 5 min in the box with three neutral objects present. The following behaviors were recorded: number of seconds in contact with the objects, number of squares traversed (locomotor activity), number of rearings, and duration of grooming in seconds. Preference for novelty was based on the difference between exploration of novel versus neutral objects, and the mean time of contact with the two neutral objects was used. 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 position had been replaced by the novel object with uneven top. Changing position of neutral object makes up a novelty in itself (Ennaceur et al., 1996). During this period of time, the same measures as in Phase 1 were made. Prior to testing of each rat the apparatus and objects were carefully washed with Neodisher GK (Miele, Germany) dissolved in water and allowed to dry. In Sessions II and III (test days 2 and 3), the same procedure was followed, and the novelty was represented by the smell of cheese on one side of the cube and a smaller object, respectively. Since changing the order of novelty presentation can lead to different patterns of locomotor and rearing activities, a counterbalanced order of testing was not used to control for accumulative effects of drugs on activity measures. The same set of neutral cubes was used after olfactory cues had properly been eliminated. Exploration of an object was defined as directing the snout toward the object at a distance of 1.5 cm or less. Bodily touch other than by the snout was not considered as exploratory behavior. One observer who was

Table 1
Mean (±SEM) measures of exploratory behavior in seconds in novelty test in Experiment 1

Group	N	Differential time exploring			Total time exploring					
		Session			Session					
		I	II	III	I		II		III	
		Ph 2	Ph 2	Ph 2	Ph 1	Ph 2	Ph 1	Ph 2	Ph 1	Ph 2
Saline	8	5.1±0.8	14.9±2.4	12.6±4.0	21.0±2.9	15.0±2.1	15.8±3.7	23.0±5.0	11.2±2.7	20.1±6.6
Benactyzine	8	0.1[a]±0.3	3.6[b]±0.7	5.9±1.6	14.4±1.5	5.5±2.9	11.3±2.3	6.0[a]±1.4	9.8±1.7	8.4±2.0
Biperiden	8	3.3±1.9	12.4±2.8	7.6±2.3	13.4±2.7	10.9±4.1	8.0±2.0	16.0±2.3	9.4±3.0	12.5±3.8
Caramiphen	8	0.8±1.2	6.6[a]±1.7	1.8[a]±1.2	18.3±3.2	3.1±1.0	6.0±2.3	10.9±3.4	9.5±3.8	7.3±3.6
Procyclidine	8	3.8±1.3	10.9±1.7	11.6±1.5	13.4±1.5	10.3±2.5	8.9±1.7	17.0±3.9	11.4±3.0	14.5±2.7
Trihexyphenidyl	8	0.3[a]±0.6	16.4±2.4	3.4[a]±1.6	15.0±2.1	10.4±3.8	14.1±4.7	18.3±3.0	10.0±2.9	13.5±5.0

Ph = Phase. Significantly different from the saline group: [a]$p<0.05$, [b]$p<0.01$.

unaware of the rats' group assignment, recorded the data manually without TV monitoring.

2.5. Statistics

Overall analyses were carried out with one-way analysis of variance (ANOVA). Group comparisons were made with Newman–Keuls post hoc test. Computations were made with Prism statistical software program (GraphPad Software CA, USA).

3. Results

3.1. Experiment 1

Decreased preference for novelty was seen among the rats treated with benactyzine, caramiphen, and trihexyphenidyl (Table 1). In Session I (uneven top of novel object), ANOVA revealed a significant treatment effect (F (5,42)=3.184, P=0.0159). The benactyzine and trihexyphenidyl groups displayed reliably less preference for novelty than the saline group ($P<0.05$). In Session II (smell novelty), ANOVA showed a reliable overall effect (F (5,42)=5.730, P=0.0004). Both the benactyzine group and caramiphen group had a preference deficit relative to the saline and trihexyphenidyl groups ($P<0.05$). The benactyzine group also performed poorer than the biperiden and procyclidine groups ($P<0.05$). In Session III (smaller object novelty), ANOVA revealed a significant treatment effect (F (5,42)=3.727, P=0.0070). The caramiphen and trihexyphenidyl groups explored the novel object reliably less than the saline group ($P<0.05$). The caramiphen group also deviated significantly from the procyclidine group ($P<0.05$).

The total time exploring objects also differed among groups (Table 1). A reliable treatment effect was seen for Phase 2 in Session II (F (5,42)=3.153, P=0.0166). The benactyzine group explored the neutral objects significantly less than the saline-treated group ($P<0.05$).

As seen from Table 2, rats treated with drugs tended to display less motor activity than the control animals. In Phase 2 in Session I, significant differences were observed among the groups (F (5,42)=2.773, P=0.0298). The caramiphen group was reliably less active than the saline group ($P<0.05$). In Phase 1 in Session II, a reliable overall effect was observed (F (5,42)=6.262, P=0.002). The benactyzine and caramiphen groups displayed reduced activity relative to the saline and trihexyphenidyl groups ($P<0.05$). The caramiphen group was also less active than the biperiden group ($P<0.05$). In Phase 2 in Session II, ANOVA showed a significant treatment effect (F (5,42)=3.173, P=0.0161). The caramiphen group was reliably less active than the saline group ($P<0.01$). In Phase 1 in Session III, a significant treatment effect was revealed (F(5,42)=4.955, P=0.0012). The benactyzine, biperiden, caramiphen, and procyclidine groups displayed reliably less activity than the saline-treated group ($P<0.05$). The caramiphen group was also less active than the trihexyphenidyl group ($P<0.05$). In Phase 2 in Session III, ANOVA showed a reliable treatment effect (F (5,42)=2.692, P=0.0338). The group treated with caramiphen was significantly less active than the saline group ($P<0.05$).

The rearing activity also differed among the groups (Table 2). In Phase 2 in Session I, a significant treatment effect was seen (F (5,42)=4.933, P=0.0012). The caramiphen group exhibited reliably less rearing than the saline group ($P<0.001$). The caramiphen group also made significantly less rearing than the benactyzine, biperiden, and trihexyphenidyl groups ($P<0.05$). In Phase 1 in Session II, ANOVA revealed a reliable overall effect (F (5,42)=7.253, $P<0.0001$). The benactyzine and caramiphen groups displayed significantly less rearing than the saline group ($P<0.05$). The caramiphen group also made reliably less rearing than the benactyzine, biperiden, procyclidine, and trihexyphenidyl groups ($P<0.05$). In Phase 2 in Session II, ANOVA showed a reliable treatment effect (F (85,42)=3.495, P=0.0099). The caramiphen group displayed significantly less rearing than the saline group ($P<0.01$). In Phase 1 in Session III, a reliable overall effect was seen (F (5,42)=7.734, $P<0.0001$). Both the benactyzine, biperiden, caramiphen, and procyclidine groups

Table 2
Mean (±SEM) measures of locomotor (squares) and rearing activities in novelty test in Experiment 1

Group	N	Squares						Rearings					
		Session						Session					
		I		II		III		I		II		III	
		Ph1	Ph 2	Ph 1	Ph 2	Ph 1	Ph 2	Ph 1	Ph 2	Ph 1	Ph 2	Ph 1	Ph 2
Saline	8	87.3±11.6	61.6±16.2	87.1±12.7	49.9±10.9	86.3±13.0	63.6±12.3	24.0±1.6	17.3±2.4	24.9±2.7	15.0±3.0	27.4±3.0	19.6±3.9
Benactyzine	8	56.9±7.5	30.3±6.7	33.9[b]±7.2	19.9±5.2	35.9[b]±8.6	26.9±10.3	18.3±1.9	11.6±1.9	15.0[a]±1.9	7.9±1.5	12.4[b]±2.5	10.3±3.0
Biperiden	8	60.1±9.4	38.5±9.9	61.3±9.8	35.4±10.4	49.8[a]±9.6	25.3±9.3	17.1±2.1	12.4±3.0	21.3±3.3	10.5±2.8	17.8[a]±3.0	9.3±3.3
Caramiphen	8	55.1±8.0	11.3[a]±4.3	18.1[c]±6.1	8.6[b]±3.2	24.3[b]±10.5	18.9[a]±8.9	16.8±2.2	3.9[c]±1.4	5.9[c]±1.7	3.8[b]±1.3	8.8[c]±2.6	6.4[a]±2.5
Procyclidine	8	93.8±8.7	37.6±7.1	51.1±9.3	32.4±7.3	46.1[a]±10.5	35.8±8.9	20.4±2.1	9.4±1.7	16.6±1.8	12.0±1.1	15.9[a]±1.6	12.8±2.5
Trihexyphenidyl	8	82.1±10.1	54.3±14.7	72.5±13.4	36.9±8.4	69.1±7.6	33.3±7.2	21.8±0.6	14.9±2.2	19.9±2.8	11.5±2.0	23.6±2.0	12.0±2.0

Ph = Phase. Significantly different from the saline group: [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$.

Table 3
Mean (±SEM) measures of exploratory behavior in seconds in novelty test in Experiment 2

Group	N	Differential time exploring			Total time exploring					
		Session			Session					
		I	II	III	I		II		III	
		Ph 2	Ph 2	Ph 2	Ph 1	Ph 2	Ph 1	Ph 2	Ph 1	Ph 2
Saline	8	2.8±1.7	20.8±3.4	17.3±2.6	25.0±2.6	17.8±3.0	14.1±1.6	30.0±5.2	15.1±3.2	28.1±3.8
Phy+Benact	8	3.3±1.8	15.4±4.5	12.6±3.2	19.0±2.1	20.0±4.5	10.9±1.7	20.0±5.6	17.0±3.9	24.9±3.5
Phy+Biperi	8	2.1±0.9	21.1±5.8	9.2±2.3	8.9^{c}±2.5	4.8±1.7	17.3±3.7	23.1±5.4	15.9±3.7	15.4±2.4
Phy+Caram	8	6.6±2.9	16.5±4.7	14.0±3.6	19.6±3.0	18.6±4.3	14.1±2.1	22.1±5.3	13.9±4.1	27.5±2.7
Phy+Procy	8	2.5±0.7	16.5±2.0	13.4±2.0	17.0±1.7	6.4±1.0	9.3±3.6	20.6±1.9	11.1±2.1	22.3±2.7
Phy+Trihex	8	2.3±1.9	21.5±4.5	15.7±3.2	8.9^{c}±2.4	13.0±4.6	7.0±2.8	26.4±4.2	11.1±2.9	30.1±5.4

Ph = Phase, Phy = physostigmine, Benact = benactyzine, Biperi = biperiden, Caram = caramiphen, Procy = procyclidine, Trihex = trihexyphenidyl. Significantly different from the saline group: $^{c}p<0.001$.

displayed significantly reduced rearing relative to the saline group ($P<0.05$). The benactyzine and caramiphen groups also performed reliably less rearing than the trihexyphenidyl-treated rats ($P<0.05$). In Phase 2 in Session III, ANOVA showed a significant treatment effect (F (5,42)=2.357, P=0.0467). The caramiphen group made reliably less rearing than the saline group ($P<0.05$).

3.2. Experiment 2

When physostigmine was combined with antiparkinson agents, preference for novelty did not differ among the groups (Table 3).

The total time exploring objects differed slightly among the groups (Table 3). ANOVA showed a significant treatment effect in Phase 1 in Session I (F(5,42)=6.950, $P<0.0001$). Both the physostigmine+biperiden and physostigmine+trihexyphenidyl groups explored the neutral objects reliably less than the saline group ($P<0.001$). The physostigmine+biperiden and physostigmine+trihexyphenidyl groups also explored significantly less than the physostigmine+benactyzine and physostigmine+caramiphen groups ($P<0.05$).

Some rats treated with combination of drugs tended to display reduced locomotor activity (Table 4). In Phase 1 in Session I, ANOVA, revealed a significant overall effect (F (5,42)=3.320, P=0.0129). The physostigmine+trihexyphenidyl group showed reliably less motor activity than the saline and physostigmine+benactyzine groups ($P<0.05$). In Phase 2 in Session I, a reliable treatment effect was observed (F (5,42)=6.315, P=0.0002). All groups treated with drugs except physostigmine+benactyzine displayed significantly less locomotor activity than the saline group ($P<0.05$). The physostigmine+biperiden, procyclidine, or trihexyphenidyl groups also displayed reduced motor activity relative to the physostigmine+benactyzine group ($P<0.05$). In Phase 1 in Session II, ANOVA showed a reliable overall effect (F (5,42)=4.041, P=0.0044). The physostigmine+trihexyphenidyl group exhibited significantly less locomotor activity than the saline group ($P<0.001$).

Table 4 shows the rearing activity among the groups. In Phase 1 in Session I, ANOVA revealed a significant overall effect (F(5,42)=10.970, $P<0.0001$). The physostigmine+biperiden or trihexyphenidyl groups made reliably less rearing than the saline group and the physostigmine+benactyzine, caramiphen, or procyclidine groups ($P<0.01$). In Phase 2 in Session I, a reliable treatment effect was found (F (5,42)= 5.872, P=0.0003). Relative to the saline group the groups treated with physostigmine+biperiden, procyclidine, or trihexyphenidyl displayed significantly reduced rearing activity ($P<0.05$). The physostigmine+biperiden group also made less rearing than the physostigmine+benactyzine or caramiphen groups ($P<0.05$). The physostigmine+trihexyphenidyl group displayed reduced rearing relative to the physostigmine+caramiphen group ($P<0.05$). In Phase 1 in Session II, ANOVA showed a significant overall effect (F (5,42)=5.830, P=0.0004). All groups treated with drugs made reliably less rearing than the saline group ($P<0.05$). In Phase 2 in Session II, a reliable treatment effect was seen (F (5,42)=2.912, P=0.0241). The physostigmine+benactyzine, biperiden, or trihexyphenidyl groups exhibited significantly reduced rearing activity compared with the saline group ($P<0.05$). In Phase 1 in Session III, ANOVA revealed a reliable treatment effect (F (5,42)=4.730, P=0.0016). All groups treated with pharmacological agents displayed reduced rearing relative to the saline group ($P<0.05$).

4. Discussion

The results from the present study demonstrated that some antiparkinson drugs like benactyzine, caramiphen, trihexyphenidyl can exert marked cognitive side effects, but when physostigmine was

Table 4
Mean (±SEM) measures of locomotor (squares) and rearing activities in novelty test in Experiment 2

Group	N	Squares						Rearings					
		Session						Session					
		I		II		III		I		II		III	
		Ph1	Ph 2	Ph 1	Ph 2	Ph 1	Ph 2	Ph 1	Ph 2	Ph 1	Ph 2	Ph 1	Ph 2
Saline	8	77.3±8.0	57.8±10.6	85.3±9.6	49.5±9.1	74.5±9.9	58.6±7.6	23.0±1.5	16.4±2.4	24.9±3.0	16.8±3.0	25.3±3.1	19.8±3.2
Phy+Benact	8	75.1±8.1	46.3±9.6	53.3±13.6	31.4±7.8	55.5±13.7	42.8±7.1	16.9±1.3	12.1±1.6	14.6^{a}±3.1	7.5^{a}±1.7	13.1^{b}±2.6	14.1±1.6
Phy+Biperi	8	56.4±9.9	20.3^{b}±3.8	50.6±12.2	32.5±8.5	55.8±11.6	38.1±9.3	9.4^{c}±2.0	3.8^{c}±1.4	12.3^{a}±3.1	8.4^{a}±2.5	10.5^{b}±2.3	8.8±1.8
Phy+Caram	8	60.9±9.9	30.1^{a}±5.7	47.1±8.8	27.8±6.2	51.9±9.9	35.4±6.1	19.4±2.1	13.5±1.8	14.8^{a}±2.6	10.6±1.5	16.3^{a}±2.9	15.0±2.0
Phy+Procy	8	62.3±5.5	16.6^{b}±2.9	46.4±7.2	28.6±6.2	58.0±11.4	30.4±7.2	18.3±1.0	8.1^{a}±2.7	14.1^{a}±1.5	11.3±1.9	15.6^{a}±1.7	11.9±2.7
Phy+Trihex	8	36.8^{a}±5.8	16.9^{b}±4.5	23.5^{c}±5.1	25.3±3.9	38.8±6.9	34.0±6.4	7.9^{c}±2.4	5.8^{b}±1.8	4.8^{c}±1.7	7.5^{a}±1.3	10.0^{b}±2.6	11.3±2.9

Ph = Phase. Phy = Physostigmine, Benact = benactyzine, Biperi = biperiden, Caram = caramiphen, Procy = procyclidine, Trihex = trihexyphenidyl. Significantly different from the saline group: $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$.

coadministered with each of these antiparkinson agents, the cognitive impairment vanished. Benactyzine, caramiphen, and trihexyphenidyl reduced preference for novelty, whereas biperiden and procyclidine did not. Furthermore, rats treated with caramiphen displayed pronounced decline in locomotor and rearing activities (Experiment 1). The combination of physostigmine and antiparkinson agents resulted in normal preference for novelty, but physostigmine combined with biperiden caused long-lasting decline in rearing activity. Physostigmine along with trihexyphenidyl produced reduction in both locomotor and rearing activities (Experiment 2).

It has been suggested that the behavioral side effects of carbamates and anticholinergics might offset each other when they are used as prophylactics to prevent nerve agent toxicity (Kim et al., 2002). This may be the case as seen from the present study, provided that the antagonism obtained by anticholinergics is completely equalized by the cholinergic agonism of physostigmine. In a previous study (Myhrer et al., 2004a), we found that the "agonistic" effect of physostigmine (0.1 mg/kg) is not sufficient to counteract cholinergic antagonism if the anticholinergic is very potent (scopolamine) or a high dose of procyclidine (6 mg/kg) is used. Additionally, if the performance level of the control group turns out to be particularly high, as in Session I in the study of Myhrer et al. (2004a), slight cognitive impairment of combined carbamate and anticholinergic may be revealed. In the latter study, it was shown that physostigmine (0.1 mg/kg) alone does not affect preference for novelty or locomotor activity. Physostigmine (0.1 mg/kg) inhibits 67% of acetylcholinesterase in the blood of rats 30 min after injection (Lennox et al., 1985). The half-life of physostigmine is 17 min in plasma and 16 min in the brain of rats. Declining concentration of physostigmine is seen up to 45 min in the brain (Somani and Khalique, 1986). In the present novelty task, the testing was terminated 40 min after injection of physostigmine and antiparkinson agents. A crucial issue is how physostigmine is able to antagonize receptor blocking effects of anticholinergics by increasing the level of acetylcholine and thus reduce or prevent cognitive impairment. This apparently subtle balance is probably attained in different ways for other relevant acetylcholinesterase inhibitors used as pretreatment against nerve agents, such as donepezil, huperzine, and galantamine.

Both cholinergic and glutamatergic antagonists produce cognitive malfunction in a number of behavioral tasks (Myhrer, 2003). Hence, it appears somewhat intriguing that coadministration with physostigmine completely compensated for the cognitive deficits caused by some antiparkinson drugs when administered alone. This finding might suggest that the deleterious impact on behavior was most prominently exerted by cholinergic antagonism. Rats injected with the glutamatergic NMDA antagonist HA-966 display reduced preference for novelty in the present task (Myhrer, 1999). However, impairment of preference for novelty that has spontaneously recovered 2–3 weeks after a combination of 2 denervations in the temporal region (fiber connections between temporal and entorhinal cortices plus hippocampal perforant path) is more effectively reactivated by atropine than HA-966 (Myhrer, 1999). Normal performance in the present novelty task might potentially be more vulnerable to cholinergic than glutamatergic antagonism.

Benactyzine, caramiphen, and trihexyphenidyl all attenuate the ability to recognize environmental change. Because the deficit was compensated for by cholinergic "agonism" (physostigmine), the nature of the deficit is probably cholinergic. Cholinergic activity has been related to attentional processes (Himmelheber et al., 2000). Thus, the cognitive impairment observed may be associated with reduced ability to make attentional shift. The total time exploring objects was not affected (except for 1 instance in rats treated with benactyzine), suggesting that the rats indiscriminately paid attention to all objects. The benactyzine group occasionally displayed decreased levels of locomotor and rearing activities. In a previous study, doses between 0.1 and 5.6 mg/kg of benactyzine do not change locomotor activity in rats (Sipos et al., 1999). When benactyzine was combined with physostigmine in the present study, a decline in rearing activity was observed. Following injection of caramiphen a pronounced and long-lasting decrease in both locomotor and rearing activities was seen. This reduction in mobility may be related to the finding of ataxia and stereotyped behavior in rats treated with 15 mg/kg of caramiphen (Szekély et al., 1994). The present caramiphen rats displayed fear and became tense when lifted in hand. Both fear and pronounced level of immobility disappeared when caramiphen was administered along with physostigmine. Trihexyphenidyl did not result in any changes in locomotor or rearing activity. Increased locomotor activity has been reported for rats injected with trihexyphenidyl doses above 5 mg/kg (Sipos et al., 1999). However, when trihexyphenidyl was combined with physostigmine, a marked decrease in rearing activity was seen in the present study.

Biperiden and procyclidine did not affect preference for novelty and only rarely reduced locomotor and rearing activities. High doses of biperiden (>5 mg/kg) produce increased locomotor activity, whereas high doses of procyclidine do not (Sipos et al., 1999). However, when procyclidine is administered in a dose of 6 mg/kg in the present novelty test, reduced preference for novelty is seen for all sessions along with moderately decreased levels of locomotor and rearing activities (Myhrer et al., 2004a). Reduced activity as expressed in locomotor and rearing may reflect declined interest in the surroundings.

The antiparkinson drugs share common anticonvulsant properties (cf. Introduction), but their side effects, as revealed in the present study, greatly differ when comparable and relatively low anticonvulsant doses are used. The combination with physostigmine further emphasizes their subtle diversity of effects. In a meta-analysis of transmitter systems and cognition in four behavioral tasks, it was concluded that tests based on innate responses (as in spontaneous alternation) appear to be more sensitive to drug-induced malfunctions than tests requiring long-lasting acquisition procedures (as radial maze) (Myhrer, 2003). Hence, the present test based on acute responding in terms of innate preference for novelty may be particularly sensitive in revealing cognitive dysfunctions.

The antiparkinson agents used in the present study have all been shown to exert good anticonvulsant efficacy against soman-induced seizures in rats when combined with pyridostigmine or physostigmine (Bajgar, 2004; Kassa et al., 2003; Kim et al., 2002; Myhrer et al., 2004b; Raveh et al., 2003). Only biperiden and procyclidine did not impair preference for novelty. However, biperiden caused more pronounced decline in rearing activity than procyclidine when these drugs were combined with physostigmine. Hence, procyclidine may be well suited for combinations with acetylcholinesterase inhibitors.

Slight cognitive impairment of effective prophylactic agents is probably inevitable. One way to circumvent the problem is to use moderate doses of prophylactics to avoid adverse impact on cognitive functions, because insufficient prophylaxis with physostigmine and procyclidine can be compensated for by adjunct treatment with scopolamine within 3 min or diazepam and pentobarbital within 15 min after seizure onset (Myhrer et al., 2004b). Procyclidine turns out to be a potent anticonvulsant with few or no cognitive side effects when used in moderate doses (Galbicka et al., 2001; Sipos et al., 2001). By testing anticonvulsant efficacy of microinfusions of antiparkinson agents into the seizure controlling region area tempestas shows that only procyclidine and caramiphen cause reliable effects (Myhrer et al., 2008). However, caramiphen appears to be associated with marked side effects as seen in this and a previous study (Szekély et al., 1994). To equalize cognitive side effects of potent anticonvulsants by use of acetylcholinesterase inhibitors is not recommendable, because subtle adjustments for differences in half-life would be required.

In conclusion, only biperiden and procyclidine did not reduce preference for novelty, whereas the cognitive impairment produced by benactyzine, caramiphen, and trihexyphenidyl could be counteracted by coadministration with physostigmine. Collectively, procyclidine turns out to be a robust anticonvulsant with few cognitive side effects either alone or in combination with physostigmine.

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