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Cognitive side effects in rats caused by pharmacological agents used to prevent soman-induced lethality

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Abstract

It is important that prophylactics used to protect military and emergency personnel against lethal doses of nerve agents do not by themselves produce impairment of cognitive capability. The purpose of the present study was to examine whether physostigmine, scopolamine, and various doses of procyclidine might reduce rats' innate preference for novelty. When these drugs were tested separately, the results showed that physostigmine (0.1 mg/kg) and procyclidine (3 mg/kg) did not affect preference for novelty, whereas scopolamine (0.15 mg/kg) and procyclidine in a higher dose (6 mg/kg) resulted in a preference deficit (Experiment 1). In Experiment 2, the combination of physostigmine and scopolamine or physostigmine and procyclidine (6 mg/kg) caused a marked deficit in preference for novelty. A much milder deficit was observed when physostigmine was combined with lower doses (1 or 3 mg/kg) of procyclidine. The latter combinations also had milder adverse impact on the animals' interest in the test environment and activity measures than the former combinations. By combining physostigmine with anticholinergics, a potentiation of adverse effects on behavior was seen. It is concluded that a slight cognitive impairment might be unavoidable with effective prophylactics.

Keywords: Nerve agent; Prophylactic; Physostigmine; Scopolamine; Procyclidine; Cognitive impairment; (Rat)

1. Introduction

Nerve agents make up a group of highly toxic organophosphates. These 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 hypersecretion, respiratory distress, tremor, seizures/convulsions, coma, and death (Taylor, 1985). 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). It takes a higher dose of anticonvulsants to terminate seizures induced by soman (pinacolyl methylphosphonofluoridate) than by other common warfare agents (Shih and McDonough, 2000). Thus, soman would be the most relevant agent to be used in animal models to evaluate potential anticonvulsant drugs, because drugs effective against soman poisoning will most likely also be effective against other nerve agents.

In order to prevent soman-induced lethality, it is important to inhibit reversibly a portion of the cholinesterase, thus protecting it from irreversible inhibition by the nerve agent. Simultaneously, there is a need to protect acetylcholine receptors by means of an anticholinergic drug. To meet these requirements, military personnel in NATO forces is currently issued with pyridostigmine (quarternary carbamate) pretreatment supported by immediate therapy with the muscarinic receptor antagonist, atropine sulphate, and an oxime to reactivate any unaged cholinesterase. However, pyridostigmine does not readily cross the blood–brain barrier (Birtley et al., 1966). On the other hand, use of physostigmine (tertiary carbamate) that readily enters the brain, along with the muscarinic receptor antagonist, scopolamine, has provided successful results with guinea pigs (Wetherell et al., 2002). Effective prevention of soman-induced lethality has also been reported for physostigmine (0.1 mg/kg) in combination with procyclidine (1–6 mg/kg)

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in guinea pigs and rats (Kim et al., 2002; Myhrer et al., 2002). Procyclidine antagonizes both muscarinic and nicotinic receptors as well as the glutamate NMDA receptor (Kim et al., 2002). Procyclidine has affinity for muscarinic M4 receptors in the rat brain (Waelbroeck et al., 1990). The anti-nicotinic properties of procyclidine are expressed in its capability to antagonize nicotinic-induced convulsions in mice (Gao et al., 1998). In a similar way, procyclidine has been shown to protect mice from the lethal effects of NMDA (McDonough and Shih, 1995).

Protection of the brain against damage caused by nerve agent poisoning requires drugs that can also affect cognitive functions. 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 been demonstrated convincingly in rats to impair the performances in Morris water maze and spontaneous alternation, whereas scopolamine also has detrimental effects on the behavior in radial maze and passive avoidance (cf., Myhrer, 2003). Hence, a crucial matter is whether the doses of prophylactics required for protection of the brain against nerve agent-induced damage will impair cognitive functions.

The purpose of the present study was to examine potential cognitive effects of a standardized dose of physostigmine and scopolamine, and various doses of procyclidine that have all been demonstrated to be effective prophylactics. Effects were tested for each drug separately (Experiment 1) and physostigmine in combination with scopolamine or procyclidine (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. Materials and methods

2.1. Animals

2.1.1. Experiment 1

Forty male Wistar rats from a commercial supplier (Møllegaard Breeding Laboratories, Denmark), weighing about 300 g when the experiment started, served as subjects. The rats were randomly assigned to one of five groups and their group assignment was unknown during testing. Eight rats received i.p. injection of saline, physostigmine, scopolamine, or procyclidine in two doses (3 and 6 mg/kg, respectively). 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 rats, weighing about 300 g when the experiment started, served as subjects. The animals were randomly assigned to the following treatment groups with eight rats in each: i.p treatment with saline (× 2), physostigmine and scopolamine, physostigmine and procyclidine (1 mg/kg), physostigmine and procyclidine (3 mg/kg), physostigmine and procyclidine (6 mg/kg). 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, scopolamine hydrobromide and procyclidine hydrochloride were purchased from Sigma (St. Louis, MO, USA). All drugs were dissolved in 0.9% physiological saline and administered i.p. in the following doses: physostigmine 0.1 mg/kg; scopolamine 0.15 mg/kg; procyclidine 1.0, 3.0, or 6.0 mg/kg. Physostigmine 0.1 mg/kg and procyclidine 1, 3, or 6 mg/kg have been reported to protect against a lethal dose of soman (Kim et al., 2002). Effective protection against soman has also been shown for a scopolamine dose of 0.156 mg/kg (Capacio and Shih, 1991). The drugs were given 20 min before each test session (1 session a day for 3 days). When physostigmine was combined with scopolamine or procyclidine (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 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, ($4.5 \times 4.5 \times 4.5$ 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 each object, 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. In Sessions II and III, the same procedure was followed, and the novelty was represented by 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 activity, a counterbalanced order of testing was not used to control for accumulative effects of drugs on activity measures. Prior to testing of each rat, the apparatus and objects were carefully washed with Neodisher (Miele, Germany) dissolved in water and allowed to dry. 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.

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 procyclidine 6 mg/kg (Table 1). In Session I (uneven top of novel object), ANOVA revealed a significant treatment effect ($F(4,35) = 7.197$, $P = 0.0002$). The procyclidine 6 (mg/kg) group displayed reliably less preference for novelty than the saline, physostigmine, scopolamine, and procyclidine 3 (mg/kg) groups ($P < 0.05$). In Session II (smell novelty), ANOVA showed a reliable overall effect ($F(4,35) = 4.943$, $P = 0.0029$). Both the scopolamine group and procyclidine 6 mg/kg group had a preference deficit relative to the saline group ($P < 0.01$). In Session III (smaller object novelty), ANOVA revealed a significant treatment effect ($F(4,35) = 8.242$, $P < 0.0001$). The scopolamine and procyclidine 6 mg/kg groups explored the novel object reliably less than the saline group ($P < 0.001$). The same groups also deviated significantly from the physostigmine and procyclidine 3 mg/kg groups ($P < 0.05$).

The total time exploring objects also differed among the groups (Table 1). A reliable treatment effect was seen for Phase 1 in Session II ($F(4,35) = 6.486$, $P < 0.0005$). All four groups treated with drugs explored the neutral objects significantly less than the saline-treated group ($P < 0.05$).

Table 1
Mean measures of exploratory behavior in seconds in Experiment 1

Group	N	Differential time exploring									Total time exploring					
		Session I			Session II			Session III			Session I		Session II		Session III	
		Neut	Nov	Diff	Neut	Nov	Diff	Neut	Nov	Diff	Ph1	Ph2	Ph1	Ph2	Ph1	Ph2
Saline	8	2.0	9.1	7.1	1.9	17.3	15.4	1.9	15.0	13.1	17.5	13.1	16.0	21.0	12.8	18.8
Phy	8	1.0	7.1	6.1	1.0	11.9	10.9	1.6	13.0	11.4	9.5	9.4	8.4[a]	13.9	7.4	16.3
Sco	8	1.9	5.0	3.1	0.6	4.9	4.3[b]	1.0	5.1	4.1[c]	12.5	9.0	3.1[c]	6.3[b]	1.6[b]	7.1
PCD 3	8	1.9	5.6	2.4	2.3	12.9	10.6	1.9	11.9	10.0	13.5	9.4	7.6[a]	18.3	13.0	15.6
PCD 6	8	2.4	1.6	−0.8[c]	1.4	5.4	4.0[c]	3.1	6.6	3.5[c]	15.8	6.0	8.5[b]	8.1[a]	4.3	12.9

Total time exploring does not match with Neut + Nov because Neut is based on the mean time of exploring two objects. Abbrevations: Diff = difference; Neut = neutral; Nov = novel; PCD = procyclidine 3 (mg/kg); Ph = phase; Phy = physostigmine; Sco = scopolamine.

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$.

In Phase 2 in Session II, ANOVA revealed a significant overall effect ($F(4,35)=4.923$, $P=0.003$). The scopolamine and procyclidine 6 mg/kg groups explored objects reliably less than the saline group ($P<0.01$ and $P<0.05$, respectively). In Phase 1 in Session III, a reliable treatment effect was observed ($F(4,35)=4.870$, $P=0.0074$). Only the scopolamine group explored the neutral objects significantly less than the saline group ($P<0.01$).

As seen from Fig. 1A, the rats treated with drugs tended to display less motor activity than the control animals. In Phase 1 in Session I, ANOVA revealed a reliable treatment effect ($F(4,35)=5.921$, $P=0.001$). Ad hoc comparisons showed that the physostigmine group was significantly less active than the saline ($P<0.01$), procyclidine 3 mg/kg ($P<0.001$), and scopolamine ($P<0.05$) groups. In Phase 2 in Session I, significant differences were observed among the groups ($F(4,35)=3.369$, $P=0.0196$). The physostigmine group was reliably less active than the scopolamine group ($P<0.01$). In Phase 1 in Session III, a significant treatment effect was revealed ($F(4,35)=7.372$, $P=0.0002$). All four groups treated with drugs displayed reliably less activity than the saline-treated group ($P<0.01$). In Phase 2 in session III, ANOVA showed a reliable treatment effect ($F(4,35)=7.446$, $P=0.0002$). All four groups treated with drugs were significantly less active than the saline group ($P<0.05$).

Fig. 1B shows the rearing activity among the groups. In Phase 1 in Session I, ANOVA revealed a significant overall effect ($F(4,35)=6.588$, $P=0.0005$). The physostigmine group displayed reliably less rearing than the saline, scopolamine, procyclidine 3 mg/kg, and procyclidine 6 mg/kg groups ($P<0.01$). In Phase 2 in Session I, a significant treatment effect was seen ($F(4,35)=9.962$, $P<0.0001$). The scopolamine group exhibited reliably more rearing than the saline, procyclidine 3 and 6 mg/kg, and physostigmine groups ($P<0.01$). The physostigmine group made signifi-

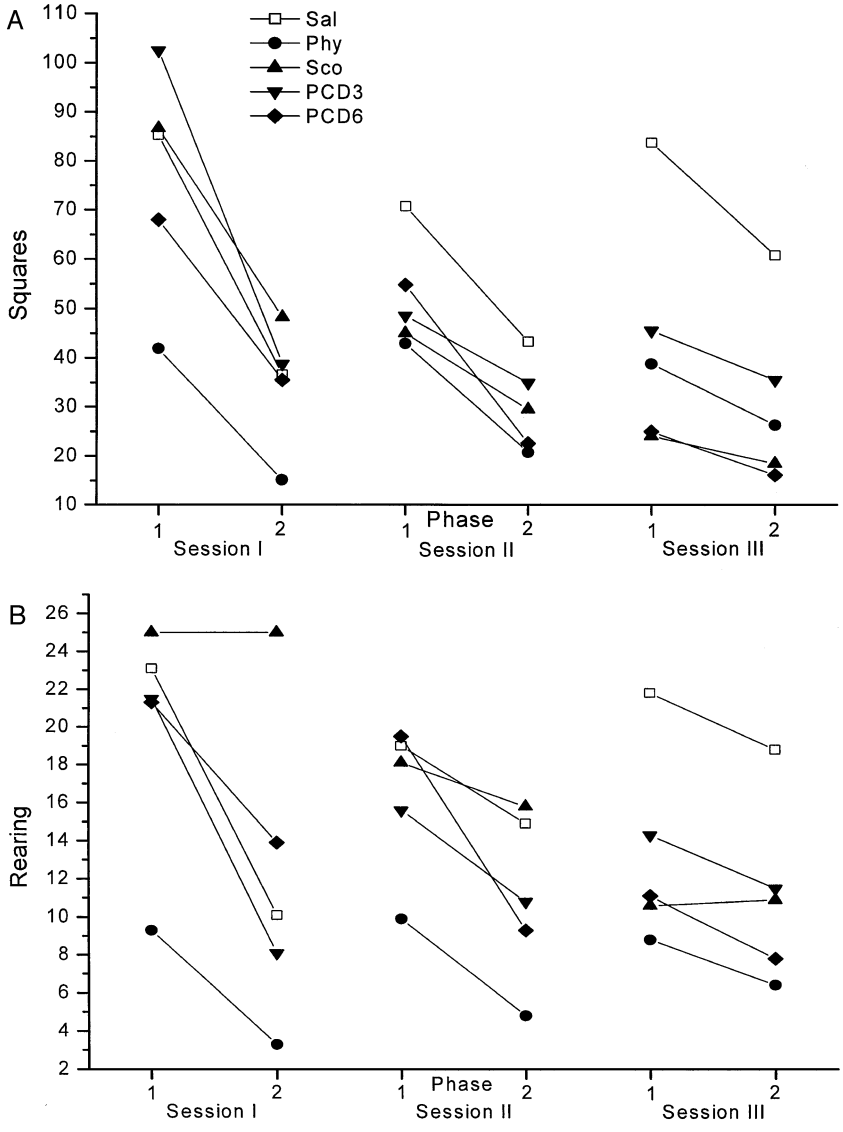

Fig. 1. Mean measures of locomotor activity (A) and rearing (B) in Experiment 1.

cantly less rearing than the procyclidine 6 mg/kg groups ($P<0.05$). In Phase 2 in Session II, ANOVA revealed a reliable overall effect ($F(4,35)=4.557$, $P=0.0046$). The physostigmine group displayed significantly less rearing than the saline and scopolamine groups ($P<0.01$). In Phase 1 in Session III, a reliable overall effect was seen ($F(4,35)=3.569$, $P=0.0153$). Both the physostigmine and procyclidine 6 mg/kg groups displayed significantly reduced rearing relative to the saline group ($P<0.05$). In Phase 2 in Session III, ANOVA showed a significant treatment effect ($F(4,35)=3.587$, $P=0.0149$). The physostigmine, scopolamine, and procyclidine 6 mg/kg groups made reliably less rearing than the saline group ($P<0.05$).

3.2. Experiment 2

In Session I, decreased preference for novelty was observed among all groups treated with drugs (Table 2). ANOVA revealed a reliable overall effect ($F(4,35)=8.159$, $P<0.0001$). All drug-treated groups displayed reduced preference for novelty relative to the saline group ($P<0.01$). In Session II, a significant treatment effect was seen ($F(4,35)=6.642$, $P=0.0004$). Both the physostigmine + scopolamine group and the physostigmine + procyclidine 6 mg/kg group had a preference deficit compared to the saline group ($P<0.01$). In Session III, ANOVA uncovered a reliable overall effect ($F(4,35)=5.272$, $P<0.0001$). The physostigmine + scopolamine group displayed significantly less preference for novelty than the saline group ($P<0.01$), the physostigmine + procyclidine 1 mg/kg group ($P<0.01$), and the physostigmine + procyclidine 3 mg/kg group ($P<0.05$). Also the physostigmine + procyclidine 6 mg/kg group payed reliably less attention to novelty than the saline group ($P<0.001$), physostigmine + procyclidine 1 mg/kg group ($P<0.001$) and physostigmine + procyclidine 3 mg/kg group ($P<0.01$).

The total time exploring objects also differed among the groups (Table 2). ANOVA showed a significant treatment effect in Phase 1 in Session I ($F(4,35)=3.528$, $P=0.0161$). Both the physostigmine + scopolamine and physostigmine + procyclidine 6 mg/kg groups explored the neutral objects reliably less than the saline group ($P<0.05$). In Phase 2 in Session I, a reliable overall effect was found ($F(4,35)=6.335$, $P=0.0006$). All groups treated with drugs exhibited significantly decrease exploration of the objects compared with the saline-treated group ($P<0.01$). In Phase 2 in Session II, ANOVA revealed a significant treatment effect ($F(4,35)=13.42$, $P<0.0001$). Relative to the saline group, the objects were reliably less inspected by the physostigmine + scopolamine group ($P<0.001$), the physostigmine + procyclidine 1 mg/kg group ($P<0.05$), and the physostimine + procyclidine 6 mg/kg group ($P<0.001$). The same groups also explored objects reliably less than the physostigmine + procyclidine 3 mg/kg group ($P<0.05$). In Phase 1 in Session III, a reliable overall effect was found ($F(4,35)=6.059$, $P=0.0008$). Compared with the saline group, significantly less exploring of neutral objects was seen among the physostigmine + scopolamine group ($P<0.001$), the physostigmine + procyclidine 1 mg/kg group ($P<0.05$), and the physostigmine + procyclidine 6 mg/kg group ($P<0.01$). The physostigmine + scopolamine group also explored objects significantly less than the physostigmine + procyclidine 3 mg/kg group ($P<0.05$). In Phase 2 in Session III, ANOVA revealed a reliable treatment effect ($F(4.35)=13.08$, $P<0.0001$). Both the physostigmine + scopolamine and physostigmine + procyclidine 6 mg/kg groups displayed significantly reduced exploration of objects compared with the saline and physostigmine + procyclidine 1 and 3 mg/kg groups ($P<0.001$).

Rats treated with combination of drugs tended to display reduced locomotor activity (Fig. 2A). In Phase 2 in Session I, ANOVA revealed a significant overall effect ($F(4,35)=3.625$, $P=0.0142$). The physostigmine + procyclidine 6 mg/kg group showed reliably less motor activity than the saline group ($P<0.05$). In Phase 2 in Session II, a reliable treatment effect was observed ($F(4,35)=6.583$, $P=0.0005$). All groups treated with drugs displayed significantly less locomotor activity than the saline group ($P<0.05$). In Phase 1 in Session III, ANOVA showed a reliable overall effect ($F(4,35)=7.487$, $P=0.0002$). Both physostigmine + scopolamine and physostigmine + procyclidine 6 mg/kg groups exhibited significantly less locomotor activity than the

Table 2
Mean measures of exploratory behavior in seconds in Experiment 2

Group	N	Differential time exploring									Total time exploring					
		Session I			Session II			Session III			Session I		Session II		Session III	
		Neut	Nov	Diff	Neut	Nov	Diff	Neut	Nov	Diff	Ph1	Ph2	Ph1	Ph2	Ph1	Ph2
Saline	8	2.9	10.8	8.0	2.4	16.6	14.3	2.4	17.5	15.1	22.1	16.6	16.4	21.4	13.9	22.3
Phy + Sco	8	1.5	0.6	−0.9[a]	0.6	6.0	5.4[a]	0.6	4.1	3.5[b]	9.5[c]	3.6[b]	3.3	7.3[a]	0.9[a]	4.8[a]
Phy + PCD1	8	1.5	4.4	2.9[b]	1.1	11.6	10.6	1.3	15.8	14.5	13.5	7.4[b]	10.9	14.0[c]	6.3[c]	18.3
Phy + PCD3	8	1.3	2.9	1.6[b]	3.9	14.1	10.3	3.4	13.0	9.6	15.0	5.4[b]	9.5	21.9	9.6	19.8
Phy + PCD6	8	0.9	0.1	−0.8[a]	0.4	5.6	5.3[b]	1.4	1.9	0.5[a]	9.1[c]	1.9[a]	8.5	5.9[a]	3.8[b]	4.6[a]

Abbreviations as for Table 1.

[a] $P<0.001$.

[b] $P<0.01$.

[c] $P<0.05$.

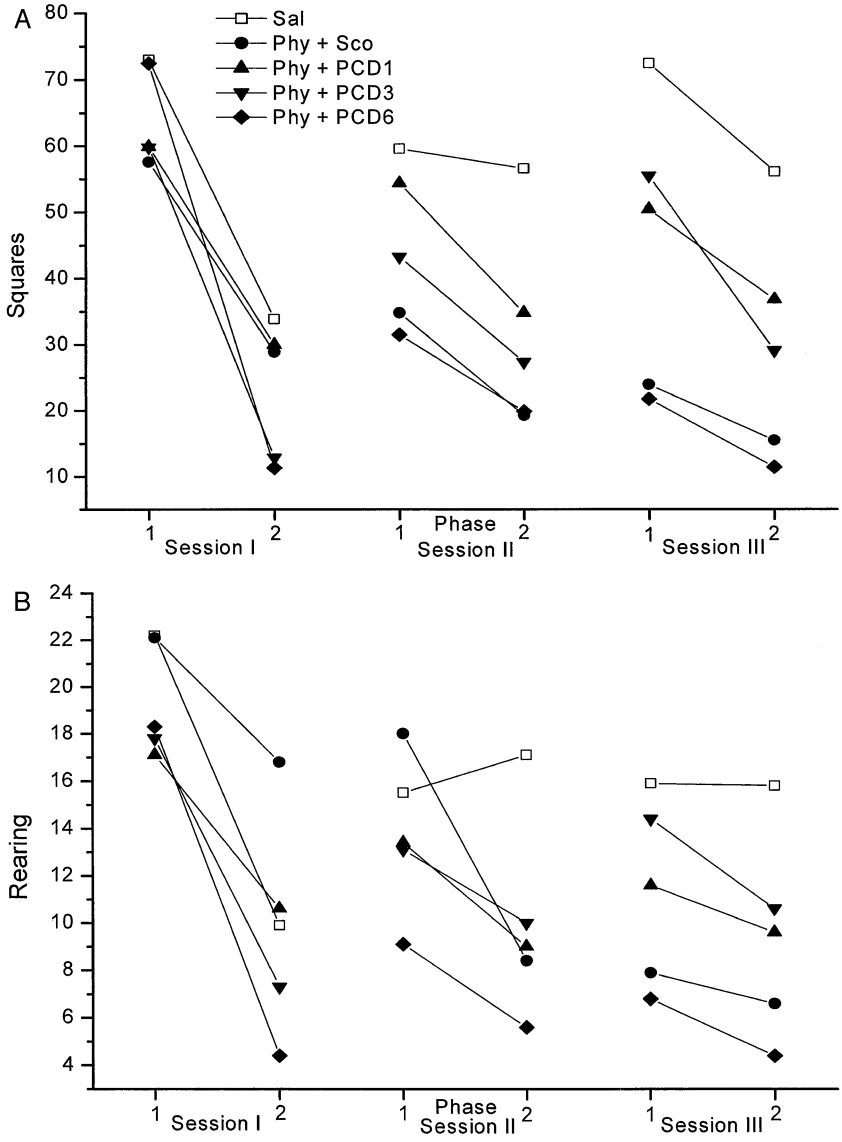

Fig. 2. Mean measures of locomotor activity (A) and rearing (B) in Experiment 2.

saline group ($P<0.001$) and the physostigmine + procyclidine 1 and 3 mg/kg groups ($P<0.05$). In Phase 2 in Session III, a reliable treatment effect was seen ($F(4,35)=6.361$, $P=0.0006$). The physostigmine + scopolamine, physostigmine + procyclidine 3 and 6 mg/kg groups displayed significantly less locomotor activity than the saline group ($P<0.05$).

Fig. 2B shows the rearing activity among the groups. In Phase 2 in Session I, ANOVA revealed a significant overall effect ($F(4,35)=3.901$, $P=0.0101$). The physostigmine + procyclidine 3 and 6 mg/kg groups made reliably less rearings than the physostigmine + scopolamine group ($P<0.05$). In Phase 2 in Session II, a reliable treatment effect was found ($F(4,35)=5.370$, $P=0.0018$). Relative to the saline group, all groups treated with drugs displayed significantly reduced rearing activity ($P<0.05$). In Phase 1 in Session III, ANOVA showed a significant overall effect ($F(4,35)=3.549$, $P=0.0156$). The physostigmine + procyclidine 6 mg/kg group made reliably less rearings than the saline group ($P<0.05$). In Phase 2 in Session III, a reliable treatment effect was seen ($F(4,35)=3.504$, $P=0.0165$). The physostigmine + scopolamine and physostigmine + procyclidine 6 mg/kg groups exhibited significantly reduced rearing activity compared with the saline group ($P<0.05$).

4. Discussion

The results from the present study clearly showed that the prophylactics tested can have marked cognitive side effects. The preference for novelty was reduced in rats that received scopolamine or 6 mg/kg of procyclidine, whereas 3 mg/kg of procyclidine had no effect (Table 3). The rats with reduced preference for novelty also displayed decreased

Table 3
Preference for novelty relative to saline-treated rats

Experiment 1	Session		
Group (mg/kg)	I	II	III
Phy 0.1	–	–	–
Sco 0.15	–	↓	↓
PCD 3.0	–	–	–
PCD 6.0	↓	↓	↓
Experiment 2	**Session**		
Group (mg/kg)	I	II	III
Phy 0.1 + Sco 0.15	↓	↓	↓
Phy 0.1 + PCD 1.0	↓	–	–
Phy 0.1 + PCD 3.0	↓	–	–
Phy 0.1 + PCD 6.0	↓	↓	↓

↓, decreased; –, unchanged.
Abbreviations as for Table 1.

interest in the surroundings (Experiment 1). The combination of physostigmine and scopolamine or physostigmine and procyclidine (6 mg/kg) resulted in a pronounced decrement in preference for novelty (Table 3) and interest in the environment. Physostigmine in combination with procyclidine in doses of 1 or 3 mg/kg caused a mild deficit in preference for novelty and interest in the surroundings (Experiment 2). The locomotor and rearing activities were also affected by the drugs to different degrees. Taken together, the data from the two experiments show that interaction in effects is not predictable from the results known for a single drug. It has been expected that possible adverse effects of carbamates and anticholinergics used prophylactically might be offset by each other (cf. Kim et al., 2002). In view of the present results, this is not the case for cognitive performance.

In rats pretreated with the oxime HI-6 ([[[(4-minocarbonyl)pyridino]methoxy]methyl]-2-[(hydroxyimino)-methyl]-pyridinium dichloride); 125 mg/kg), the lowest effective dose of scopolamine protecting against soman-induced seizures is 0.156 mg/kg (Capacio and Shih, 1991). For this reason, 0.15 mg/kg of scopolamine was chosen as relevant dose for comparisons with effects of various doses of procyclidine. Physostigmine (0.1 mg/kg) alone is able to reduce convulsant effects of soman (Kim et al., 2002). Physostigmine (0.1 mg/kg) in combination with procyclidine (1–6 mg/kg) has been shown to protect rats with an increasing degree of confidence against a soman dose of $1.3 \times LD_{50}$ (Kim et al., 2002). Furthermore, physostigmine (0.1 mg/kg) combined with procyclidine doses of 1, 3, or 6 mg/kg prevent seizures in rats when the soman doses are 1.3, 1.6, or $2.0 \times LD_{50}$, respectively (Myhrer et al., 2002). Thus, the doses and combinations of drugs tested in the present study correspond with those previously found to be effective in prophylaxis.

The effects obtained with the present drugs can be interpreted in various ways. Physostigmine in a low dose (0.03 mg/kg) has been reported to enhance passive avoidance performance in rats (Santucci et al., 1989). A physostigmine dose of 0.1 mg/kg improves radial maze performance (Ennaceur, 1998), whereas a dose of 0.05 mg/kg does not affect the performance in the same task (Cassel and Kelche, 1989). Furthermore, a dose of 0.2 mg/kg physostigmine impairs discrimination between novel and familiar objects (Ennaceur and Meliani, 1992). Physostigmine (0.125 mg/kg) does not affect working memory in operant continuous delayed response, whereas higher doses decrease responding indiscriminately (Heise and Hudson, 1985). The impairing effect obtained with higher doses of physostigmine may be due to an excessive presence of acetylcholine leading to a blockade rather than a facilitation of neurotransmission (cf., Ennaceur, 1998). The present dose of 0.1 mg/kg had no effect on preference for novelty, but reduced locomotor and rearing activity. The physostigmine dose of 0.1 mg/kg inhibits 67% of the acetylcholinesterase in the blood of rats 30 min after injection (Lennox et al., 1985). A corresponding cholinesterase inhibition is obtained with 28 μg i.v. in humans (D'Mello and Sidell, 1991).

Effects of scopolamine on water maze performance have been tested with doses of 0.1–1.0 mg/kg in rats (McNamara and Skelton, 1993). Scopolamine has indisputable detrimental effect on the performance of rats in water maze, radial maze, passive avoidance, and spontaneous alternation (Myhrer, 2003). In behavioral studies, the dose of scopolamine commonly used is 0.5 mg/kg (Andersen et al., 2002), whereas cognitive impairment was found with a dose of 0.15 mg/kg in the present study. However, scopolamine has been reported to impair performance in delayed matching to position tasks at doses as low as 0.05 and 0.075 mg/kg in rats (Kirkby et al., 1995; Stanhope et al., 1995). Whether the present novelty test is sensitive to such low doses has not been examined. Memory impairment is seen after 0.5 mg of scopolamine given i.v. in humans (Jones et al., 1991). The decreased preference for novelty seen among the present scopolamine-treated rats may be related to diminished attention (Blokland, 1996). Since these rats also inspected neutral objects less than the control animals, an attentional deficit may explain their low interest in the test environment in general. It cannot be excluded, however, that non-cognitive factors like visual deficit or motor dysfunction may be associated with the results.

Cognitive effects of procyclidine have barely been tested in previous studies. Procyclidine in a dose range of 0.1–5.6 mg/kg does not affect acoustic startle response in rats (Sipos et al., 2001). Procyclidine in a dose of 1 mg/kg does not decrease performance in an operant conditioning task, whereas higher doses impair behavior (Galbicka et al., 2001). The hippocampal theta rhythm is unaffected by procyclidine (6 mg/kg), whereas the functional NMDA antagonist HA-966 (3-amino-1-hydroxy-2-pyrrolidinone; 60 mg/kg) disrupts the theta rhythm (Myhrer et al., 2003), suggesting a weak impact of procyclidine on behavioral processes. Procyclidine has been considered to be among

the promising anticonvulsant candidates in prophylaxis because of apparently modest cognitive side effects (Galbicka et al., 2001). Only the highest dose of procyclidine (6 mg/kg) impaired cognition in the present study. This drug possesses abilities to antagonize both cholinergic and glutamatergic activity (Kim et al., 2002), and the detrimental effects seen on preference for novelty might, therefore, be associated with reduced attention as well as reduced associative activity. Humans receiving 10-mg procyclidine i.v. felt muzzy within 15 min of injection (Whiteman et al., 1985).

It has been reported that general locomotor activity can be depressed following administration of acetylcholinersterase inhibitors such as physostigmine (Frances and Jacob, 1971). In contrast, classical acetylcholine receptor antagonists like scopolamine and atropine generally increase measures of activity in many species, including the rat (Sipos et al., 1999; Walters and Block, 1969). Procyclidine has modest effects on activity in the home cage during 23-h recording, inasmuch as locomotor activity is unchanged, but grooming and scratching are increased during the first 4 h (Sipos et al., 1999). In the present study, physostigmine tended to reduce both locomotor and rearing activity, whereas scopolamine only initially increased the activity (cf., Fig. 1). During the test sessions, all drugs administered resulted in declined locomotor and rearing activity. Whether the latter finding is associated with accumulative effects of the drugs (given 20 min before each test session) is not known. The half-life in plasma of the rat after systemic administration is 17 min for physostigmine (Somani and Khalique, 1986), 17 min for scopolamine (Lyeth et al., 1992) and about 2 h for procyclidine as calculated from Fig. 4 of Jang et al. (2001). From these results the most pronounced accumulative effect would be expected for procyclidine and in particular with the highest dose. When physostigmine is combined with scopolamine or procyclidine in various doses, the outcome in terms of activity levels is unpredictable from the results seen in Experiment 1 (Fig. 1 vs. Fig. 2).

The interaction effects on preference for novelty are also unpredictable from the results seen in Experiment 1. Whenever physostigmine was combined with another prophylactic, more pronounced adverse effects were seen compared to the effects obtained with the same drugs and doses separately. The adverse effects produced by combinational prophylaxis are quite contrary to the equalizing effects expected from combining a cholinesterase inhibitor and an anticholinergic drug (Kim et al., 2002). The effects observed in the present study are not readily accounted for. A possible explanation of the adverse combinational effects might be that continuous stimulation of acetylcholine receptors resulted in desensitization and enhanced effects of the anticholinergic drugs. The combined treatment of physostigmine and scopolamine or the highest dose of procyclidine (6 mg/kg) impaired preference for novelty and additionally reduced the total time exploring objects and the levels of locomotion and rearing. The latter combinations appear to have marked detrimental effects on behavioral functions, whereas physostigmine combined with procyclidine in lower doses (1 and 3 mg/kg) produced much milder interference with behavioral functions.

Behavioral tasks possess different abilities to reveal cognitive deficits following administration of pharmacological agents. In a recent meta-analysis of transmitter systems and cognition, it was shown that the highest sensitivity (percentage of significant effects) to interference with the classical neurochemical systems was found for spontaneous alternation (86%), followed by water maze (76%), passive avoidance (72%), and radial maze (58%; Myhrer, 2003). Behavioral tests based on innate responses (as in spontaneous alternation) seem to be more sensitive to drug-induced malfunctions than tests requiring long-lasting acquisition procedures (as in radial maze). The behavioral test used in the present study is based on an innate preference for novelty. This measure of acute responding may explain the apparently high sensitivity of the novelty test in revealing cognitive dysfunctions.

In conclusion, the combination of physostigmine and scopolamine or physostigmine and a high dose of procyclidine (6 mg/kg) is not well suited as a prophylactic regimen against soman-induced lethality. On the other hand, physostigmine and procyclidine in a dose of 3 mg/kg (with markedly less adverse effects) protect effectively against a soman dose of $1.6 \times LD_{50}$ (Myhrer et al., 2002). Thus, the latter combination should be preferred above physostigmine and scopolamine. A slight cognitive impairment of prophylactic agents is probably inevitable, unless alternative and more efficient medical treatments are developed.

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