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Prophylactic potential of memantine against soman poisoning in rats

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ABSTRACT

Background: Carbamates physostigmine and pyridostigmine have been used as a pretreatment against poisoning with nerve agents in order to reversibly inhibit and thus protect from irreversible inhibition a portion of acetylcholinesterase (AChE) in brain and respiratory muscles that is crucial for survival. Memantine, an adamantine derivative, has emerged as a promising alternative to carbamates, since it prevented the fasciculations and skeletal muscle necrosis induced by carbamates and organophosphates, including nerve agents.

Aim: This experimental study was undertaken in order to investigate and compare the protective and behavioural effects of memantine and standard carbamates physostigmine and pyridostigmine in rats poisoned with soman and treated with atropine, oxime HI-6 and diazepam. Another goal was to elucidate the mechanisms of the antidotal effect of memantine and its potential synergism with standard antidotes against nerve agents.

Materials and methods: Male Wistar rats were used throughout the experiments. In dose-finding experiments memantine was administered at dose interval 0–72 mg/kg *sc* 60 min before *sc* injection of soman. In time-finding experiments memantine was injected 18 mg/kg *sc* 0–1440 min before soman. Standard treatment antidotes - atropine 10 mg/kg, HI-6 50 mg/kg and diazepam 2.5 mg/kg – were administered *im* within 15 s post-exposure. Soman 0.75 LD_{50} was used to study its inhibitions of neuromuscular transmission on the phrenic nerve-diaphragm preparation *in situ* and of tissue AChE activity. Behavioural effects of the prophylactic antidotes were investigated by means of the rotarod test. Based on these data therapeutic index and therapeutic width was calculated for all three prophylactic agents.

Results: Memantine pretreatment (18 mg/kg *sc*) produced in rats poisoned with soman significantly better protective ratios (PRs) than the two carbamates – 1.25 when administered alone and 2.3 when combined with atropine pretreatment and 6.33 and 7.23 with atropine/HI-6 and atropine/HI-6/diazepam post-exposure therapy, respectively. The highest PR of 10.11 obtained in Atr/HI-6-treated rats was achieved after pretreatment with memantine 36 mg/kg. This additional protection lasted for 8 h. All three prophylactic regimens antagonised the soman-induced neuromuscular blockade, but the effect of memantine was fastest. Pretreatment with memantine assured higher AChE activity in brain and diaphragm than in unpretreated rats (46% vs 28% and 68% vs. 38%, respectively). All three prophylactic regimens affected the rotarod performance in rats, but the effect of memantine was relatively strongest. Memantine and pyridostigmine had lowest and highest therapeutic index and therapeutic width, respectively.

Conclusions: Although memantine assures better and longer-lasting protection against soman poisoning in rats than the two carbamates, its small therapeutic index and narrow therapeutic width seriously limit its potential as a pretreatment agent. Despite its behavioural effects, memantine seems to be beneficial antidote when administered after soman, along with atropine/HI-6/diazepam therapy. Mechanism of the antidotal effect of memantine against soman poisoning appears to be a combination of AChE-protecting and NMDA receptor-blocking action.

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1. Introduction

Use of standard antidotes – atropine, oximes and diazepam – in the treatment of experimental intoxications with sarin and VX assures high protective ratios (PRs). For example, in guinea pigs poisoned with sarin or VX therapy with atropine and oxime pyridinium methanesulphonate (P2S) resulted in PRs of 38 and 25, respectively (Leadbeater, 1988). Much more serious problem represent intoxications with tabun or soman, where, under the same conditions, very modest PRs were obtained – 2.5 and 1.3, respectively (Leadbeater, 1988). It must be pointed out, though, that P2S is not an oxime that can reactivate acetylcholinesterase (AChE) inhibited by tabun or soman (Bošković et al., 1984; Ćetković et al., 1984). Trimedoxime (TMB-4), obidoxime (LüH-6), or, since recently oxime K203 are much better oximes against tabun poisonings (O'Leary et al., 1961; Inns and Leadbeater, 1983; Kuca et al., 2018), while oxime HI-6 achieves best results in rodents intoxicated with soman (Bošković, 1981; Clement and Lockwood, 1982; Shih, 1993; Antonijević and Stojiljković, 2007). If we compare the PRs in rats treated with atropine and appropriate oxime intoxicated with various nerve agents, it appears that PRs obtained in animals poisoned with sarin or VX are still 5–15 times higher that the ones after poisoning with tabun or soman (Jokanović and Stojiljković, 2006; Stojiljković and Jokanović, 2006).

It is estimated that even the well-trained military personnel can be exposed to 5 median lethal doses (LD_{50}) of soman and this is why it is expected from the antidotes to be able to reach this level of protection, i.e. PR 5 or higher (Dunn and Sidell, 1989). Having in mind the mentioned results for tabun and soman, the use of atropine and oxime treatment cannot meet this requirement and this is why this treatment after exposure to soman will not afford protection against more than 1.5 or 2 LD_{50} (Rickett et al., 1987). This is the main reason why the concept of adding a prophylactic antidote had to be conceived.

Koster (1946) was first to report on the favourable interaction between a carbamate – physostigmine – and an organophosphate (OP) AChE inhibitor – diisopropylfluorophosphate (DFP). He successfully used physostigmine as pretreatment in cats exposed with DFP and protected cats from dying after *iv* administration of 30 LD_{50} of DFP. Pretreatment regimen included a large dose of physostigmine (1 mg/kg) and a small dose of atropine (0.3 mg/kg) that was added to antagonise muscarinic effects of physostigmine. The pretreatment interval was 3.5 h. Thereafter, Koelle (1946) demonstrated that physostigmine protected AChE from irreversible inhibition by DFP in rat brain homogenates *in vitro* and formulated that this finding is the reason for Koster's phenomenon.

Fleisher and Harris (1965) discovered that it was dealkylation of the soman-AChE complex that made it resistant to reactivation by oximes – the phenomenon known as aging of the AChE-soman complex. In this study the $t_{1/2}$ of aging of the soman-AChE complex *in vitro* was only 2.2 min. The authors clearly showed that physostigmine 1 mg/kg *iv* injected 5 min after atropine 10 mg/kg *iv* and 15 min before soman *sc* assured PR of 3.8 (relative to atropine alone). They also showed that after the challenge with soman 1.5 LD_{50} *sc*, all the rats pretreated with physostigmine survived and were without any symptoms of poisoning, in comparison only 50% of those animals pretreated with atropine alone. The difference in brain AChE activity 24 h after soman challenge between these two groups was also significant – 58.5% vs 15.4%, respectively. What is even more important, it was shown that the aging reached maximum 30 min after soman intoxication, when in unpretreated rats 84.6% of all AChE activity was inhibited, out of which 50% belong to the aged portion of the enzyme, while in rats pretreated with physostigmine these percentages were much lower – 41.5% and 6%, respectively. The authors concluded that the preventing by physostigmine of the interaction between soman and AChE is a very successful way to avoid not only its inhibition by soman, but also to decrease the aging of the soman-AChE complex (Fleisher and Harris, 1965).

Further investigation into the field of potential prophylactic antidotes ensued and best protection was obtained with pyridostigmine in guinea pigs (Berry and Davies, 1970; Gordon et al., 1978). Definite affirmation pyridostigmine prophylaxis won when in experiments in guinea pigs poisoned with tabun, sarin, soman or VX and treated with atropine, various oximes and diazepam PRs of 76, 380, 20 and 410 were obtained, respectively (Inns and Leadbeater, 1983).

In the essence of this concept is reversible inhibition of a portion of AChE by a carbamate, which protects these molecules of AChE from being irreversibly inhibited by DFP or any other nerve agent (Koelle, 1946). In this case, inhibition of the remaining AChE activity by an organophosphorus compound (OPC) makes no life-threatening situation, since over the time carbamate dissociates – decarbamylates the active centre of AChE, in quantities sufficient to maintain normal transmission in cholinergic synapses (Dirnhuber and Green, 1978). The opposite sequence of administration –OPC first and carbamate second – only potentiates the cholinergic toxic effects (Koster, 1946; Takahashi et al., 1987), since carbamate inhibits the portion of AChE that remained uninhibited by the OPC (Green, 1983).

Until now, more than fifty compounds have been investigated as potential prophylactic agents against poisoning with nerve agents. Some of them have been abandoned, others are still under the investigation, while some of them were introduced in the corresponding equipment of modern armies. Survey of such medicines is contained in Table 1.

1.1. Use of AChE inhibitors in prophylaxis of poisoning with nerve agents

The most successful prophylactic schemes include carbamate AChE inhibitors physostigmine (Koster, 1946; Berry et al., 1971; Harris and Stitcher, 1984; von Bredow et al., 1991b), neostigmine (Berry et al., 1971; Heyl et al., 1980), pyridostigmine (Berry et al., 1971; Dirnhuber et al., 1979; Hauser et al., 1981; Inns and Leadbeater, 1983; Harris and Stitcher, 1984; Maxwell et al., 1988; von Bredow et al., 1991a) and some newer synthetic carbamates, like ferrocene carbamate (Gordon et al., 1978; Karlsson et al., 1984).

1.1.1. Physostigmine

Physostigmine, also called eserine, is a N-monomethyl carbamate isolated from the Calabar bean (*Physostigma venenosum* Balfour) by Jobst and Hesse (1864) and *de novo* synthesised by Julian and Pikl (1935). Physostigmine reversibly inhibits AChE in mammalian peripheral tissues and in the brain (Deyi et al., 1981; Harris and Stitcher, 1984). As a consequence, when administered as a triple prophylactic regimen with atropine and mecamylamine, it provides protection against 2.6 LD_{50} of soman in rats (Harris et al., 1980). Under the same conditions, this alkaloid assures PR of 6.9 against DFP poisoning in rats (Harris and Stitcher, 1984). With the addition of atropine alone or with atropine and P2S, physostigmine pretreatment exerts similar efficacy in other species intoxicated with soman, and especially in guinea pigs, where the attained PR of the triple prophylactic regimen reaches 10.7 (Berry et al., 1971). It was confirmed that this antidotal effect is based on the protection of AChE in vital organs from soman-induced irreversible inhibition (Deyi et al., 1981).

1.1.2. Neostigmine

Neostigmine is a quaternary *N,N*-dimethyl carbamate synthesised by Aeschlimann and Reinert (1931) as a physostigmine analogue. Neostigmine soon, due to lack of central effects, replaced physostigmine in the treatment of myasthenia gravis (Remen, 1932; Walker, 1934a,1934b). Although its hydrophilicity and exclusively peripheral action dictated its use with central and peripheral antimuscarinic atropine and predominantly central antinicotinic mecamylamine, neostigmine assured good results in prophylaxis of soman intoxications (Harris et al., 1980). From the practical point of view, neostigmine is inferior to also peripherally acting carbamate pyridostigmine because of stronger stimulation of digestive tract, which is unfavourable for a

Table 1
Prophylactic agents against organophosphorus compounds (OPC).

Mechanism of action	Class of agents	Prophylactic agent	Reference
Reversible AChE inhibition	Carbamates	Physostigmine, pyridostigmine	Dirnhuber et al. (1979); Heyl et al. (1980); Deyi et al. (1981); Inns and Leadbeater (1983); Harris et al. (1984), Leadbeater et al. (1985); Lennox et al. (1985); Shiloff and Clement (1986); Solana et al. (1990a, 1990b), von Bredow et al. (1991a, 1991b)
		Mobam, decarbofuran, neostigmine	Gordon et al. (1978); Harris et al. (1980); Heyl et al (1980)
		Ferrocene carbamate	Karlsson et al. (1984)
	Aminophenols	Eseroline	Galli et al. (1985)
	Aminoacrydines	Tetrahydroamminoacridine	Galli and Mori (1991)
	Analgesics	Meptazinol	Galli and Mazri (1988)
	Plant alkaloids	Huperzine A, galantamine	Albuquerque et al. (2006); Haigh et al. (2008); Mamczarz et al. (2011); Wang et al. (2011); Hamilton et al. (2017)
	Noncompetitive AChE inhibitors	Donepezil	Janowsky et al. (2005)
Irreverible, oxime-sensitive AChE inhibition	OPC	Tetraethylpyrophosphate	Berry et al. (1971)
		Paraoxon	
		Ethyl-4-nitrophenylmethylphosphonate	
Blockade of cholinoceptors	Antimuscarinics	Atropine	DeCandole and McPhail (1957)
		Aprophen	Leadbeater et al. (1985)
		Azaprophen	Gennings et al. (1990)
		Scopolamine	Anderson et al. (1991); Lim et al. (1991); von Bredow et al. (1991b)
		Trihexyphenydyl	Berry et al. (1971); Kassa and Vachek (2002)
		Benactyzine	Heyl et al. (1980); Kassa and Vachek (2002)
	Antinicotinics	Pentamethonium	Berry et al. (1971)
		Mecamylamine	Heyl et al. (1980); Harris et al. (1980, 1984)
		d-tubocurarine	Patterson et al. (1988)
Decreased synthesis/release of acetylcholine	Quinuclidines	N-allyl-3-quinuclidinol	Sterling et al. (1988)
		Clonidine	Aronstam et al. (1986)
AChE reactivators	Oximes	Pralidoxime salts	Crook et al. (1962); Quinby (1968); Wolthuis et al. (1981)
		Pro-2-PAM	Clement (1979)
		Obidoxime	Schoene et al. (1985)
		HI-6	Schoene et al. (1985); Bokonjić et al. (1987); Shih et al. (1991); Bajgar (2004); Bajgar et al. (2009)
		K027, K034, K048	Lucić Vrdoljak et al. (2006); Lorke and Petroianu (2018)
Treatment of convulsions	Benzodiazepines	Diazapam	Lundy et al. (1978); Doebler et al. (1985)
		Clonazepam	Lipp (1974)
Stoichiometric scavengers	Esterases	Acetylcholinesterase	Wolfe et al. (1987); Maxwell et al. (1992)
		Butyrylcholinesterase	Raveh et al. (1993); Allon et al. (1998); Ševalova et al. (2004); Saxena et al. (2011, 2015), Rosenberg et al. (2014); Reed et al. (2017)
	Immunoglobulines	Monoclonal antibodies	Lenz et al. (1984); Rong and Zhang (1990)
Catalytic scavengers	OPC bioscavengers	Paraoxonase-1 (PON-1)	Lenz et al. (2007); diTargiani et al. (2010); Valiyaveettil et al. (2011, 2012), Kuca et al. (2013)
		Prolidase	Endo et al. (1988); diTargiani et al. (2010); Kuca et al. (2013); Iyer et al. (2015)
		Senescence marker protein-30 (SMP-30)	Kondo et al. (2004); diTargiani et al. (2010); Kuca et al. (2013); Iyer et al. (2015)
		Phosphotriesterase	Tuovinen et al. (1996); Petrikovics et al. (2000); Kuca et al. (2013); Iyer et al. (2015); Poirier et al. (2018)
Protection of AChE/blockade of glutamate receptors	NMDA antagonists	Ketamine	Clinton et al. (1988)
		Memantine	Gupta and Dettbarn (1992); McLean et al. (1992),
		Dizocilpine (MK-801)	Braitman and Sparenborg (1989); Löscher and Hönack (1994); Shih et al. (1991a); Sparenborg et al. (1992)
	AMPA antagonists	NBQX	Löscher and Hönack (1994)

prophylactic agent.

1.1.3. Pyridostigmine

Pyridostigmine is an analogue of neostigmine, also with *N,N*-dimethyl carbamate structure, sinthesised by Urban and Schnider in 1945 (Randall et al., 1955). Frequency and severity of cholinergic adverse effects (i.e. nausea, vomiting, increased salivation, diarrhoea and abdominal cramps) of pyridostigmine is lower than in case of neostigmine (Duphar, 2019). Although pyridostigmine also does not penetrate the brain (Birtley et al., 1966), it is among the potential prophylactic agents by far the most tested one, owing to its much longer prophylactic interval (4 h) than physostigmine (Gordon et al., 1978). If administered 30 min before the nerve agent and if supplemented by the post-exposure therapy consisting of atropine and oxime P2S, pyridostigmine produces PRs of 22, 21.5, 8 and 26.3 in guinea pigs poisoned with tabun, sarin, soman or VX (Gordon et al., 1978). Pyridostigmine pretreatment 0.2 mg/kg and post-exposure treatment with atropine protects monkeys against 28 LD_{50} of soman (Dirnhuber et al., 1979). In guinea pigs poisoned with nerve agents best protection was provided by pretreatment with pyridostigmine and treatment with atropine, oxime and diazepam. Extremely high PRs were thus obtained – for tabun 76, for sarin 370, for soman 20 and for VX 410 (Inns and Leadbeater, 1983).

1.2. Use of memantine in prophylaxis against OPCs

Memantine hydrochloride (1-amino-3,5-dimethylaminoadamantane) is adamantine derivative used in various conditions affecting central nervous system (CNS) (Wesemann et al., 1983). It acts as a non-competitive antagonist of N-methyl-D-aspartate (NMDA) receptors for

excitotoxic transmitter glutamate (Bormann, 1989; Kornhuber et al., 1989, 1991), which explains its neuroprotective effect (Seif el Nasr et al., 1990; Erdö and Shäfer, 1991). Gupta and coworkers in late 1980s started a series of experiments, where memantine 18 mg/kg *sc* was successfully used along with atropine 16 mg/kg *sc* as pretreatment against convulsions, muscle necrosis and death induced by intoxications with tabun, sarin, soman or VX (Gupta and Dettbarn, 1992; McLean et al., 1992). It was shown that memantine pretreatment decreased four times the ED_{50} of HI-6 for anti-lethal effect in mice poisoned with soman, by counteracting the soman-induced convulsive activity (Antonijević et al., 2011). In all these publications, however, no attempt was done to ascertain PRs of memantine; instead, the effect on antagonising the specific signs and biochemical consequences of nerve agent poisoning was performed.

1.3. Aim

This experimental study was undertaken in order to investigate and compare the protective and behavioural effects of memantine, as promising new prophylactic antidote, and standard carbamates physostigmine and pyridostigmine in rats poisoned with soman and treated with atropine, oxime HI-6 and diazepam. Another goal was to elucidate the mechanisms of the antidotal effect of memantine and its potential synergism with standard antidotes against nerve agents.

2. Methods

2.1. Experimental animals

This study was approved by the local Institutional Animal Care and Use Committee. All experiments were performed in male Wistar rats weighing 200–300 g. Animals were housed under the standard conditions and given access to food and water *ad libitum*.

2.2. Chemicals

Soman of minimum 95% purity were synthetised at the Military Technical Institute, Belgrade, Serbia. Bispyridinium oxime HI-6 dichloride monohydrate was synthesised at the SBS Institute, Sarajevo, Bosnia & Herzegovina. Memantine hydrochloride (1-ammino-3,5-dimethyl adamantine) was a generous gift from Dr. G. Quack of Merz & Co. GmbH, Germany. The remaining chemicals were obtained from commercial sources.

2.3. Experimental procedures

Memantine, soman, decamethonium, and d-tubocurarine were injected *sc*, while physostigmine, pyridostigmine, atropine, HI-6 and diazepam were administered *im*. When given as a pretreatment, atropine was administered *sc*. In the *in situ* experiments, urethane was injected *ip*.

In the studies of prophylactic efficacy, various doses of memantine, physostigmine or pyridostigmine were used at different time intervals (up to 24 h) before intoxication with soman and treatment with atropine and HI-6. Protective ratios (ratio of LD_{50} value in treated rats and in untreated rats) were taken as indices of antidotal efficacy of various prophylactic and therapeutic combinations. Number of animals used per each dose of soman was kept to a minimum of three.

Memantine was administered *sc*, based on the previous experiments (Gupta et al., 1987; Patterson et al., 1988; Gupta and Dettbarn, 1992; McLean et al., 1992) and for the same reason in the majority of the experiments prophylactic regimen consisted of memantine 18 mg/kg and atropine 16 mg/kg, administered 60 and 15 min before soman, respectively.

Physostigmine 0.1 mg/kg *im* is considered as the highest asymptomatic dose of this carbamate in rats that were not co-pretreated with atropine (Berry and Davies, 1970). The same dose of physostigmine (Berry and Davies, 1970; Kawabuchi et al., 1988, 1989; Leadbeater et al., 1985; Deshpande et al., 2019) or pyridostigmine (Gordon et al., 1978; Inns and Leadbeater, 1983; Leadbeater et al., 1985) was used previously with success as a pretreatment in rodents acutely intoxicated with organophosphate AChE inhibitors. For these reasons, in the present experiment the dose of 0.1 mg/kg *im* was chosen for both physostigmine and pyridostigmine.

In biochemical experiments, animals were intoxicated with 0.75 LD_{50} of soman (50 μg/kg *sc*), which was preceded by prophylactic agents, memantine (18 mg/kg *sc*, 60 min before soman), physostigmine or pyridostigmine (both 0.1 mg/kg *im*, 30 min before soman). The activity of acetylcholinesterase (AChE) in brain and diaphragm was determined spectrophotometrically according to the slight modification (Wilhelm, 1968) of the original method (Ellman et al., 1961) and in erythrocytes titrimetrically according to Augustinsson (1971). The latter procedure was used for erythrocytes instead of the Ellman's method because the yellow TNB^{2-} dianion that occurs after cleavage of the disulphide bond in the Ellman's reagent 5,5′-dithiobis-(2-nitrobenzoic acid) or DTNB cannot be detected spectrophotometrically in the presence of red pigment of haemoglobin from erythrocytes.

For the *in situ* experiments rats were anaesthetised with 25%-urethane 7 ml/kg *ip* and prepared for registration of the indirectly evoked contractions of diaphragm according to the method of Ćetković and Bošković (1988). Animals were intoxicated with 0.75 LD_{50} of soman, which was preceded by prophylactic agents, memantine (18 mg/kg *sc*, 60 min before soman), or physostigmine or pyridostigmine (both 0.1 mg/kg *im*, 30 min before soman). Amplitudes of the contraction of their diaphragms were registered.

Test performed for the estimation of muscular tonus and movement coordination was a slight modification of the original rotarod test (Sofia, 1969). Briefly, rats were trained over three days to maintain their balance on the rotating rod (rotarod) that revolved 6 cycles per min. Whenever the animal would endure 180 s without losing its balance, the test would be terminated. The rats that failed to do so were eliminated from further testing. The remaining animals, grouped in five, were injected with prophylactic doses of memantine, physostigmine or pyridostigmine and re-tested after 30, 60 and 90 min. Proportion of rats failing to withstand the 180 s on the rotarod was used for calculation of ED_{50} by means of probit analysis (Litchfield and Wilcoxon, 1949).

2.4. Ethics

All the experiments were carried out according to the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

2.5. Statistical analysis

Analysis of variance (ANOVA) and post-hoc Tukey test were used for comparisons, with probability $p < 0.05$ being considered significant.

3. Results

In the initial experiment, previously published pretreatment regimens (Gupta and Dettbarn, 1992) of memantine (18 mg/kg sc, 60 min before soman) or/and atropine (16 mg/kg *sc*, 15 min before soman) were checked in rats. Both memantine (by 25%) and atropine (by 35%) assured significant increase in the 24-hour LD_{50} of soman protection against the soman-induced lethal effect, but the combined treatment assured even by 70% higher LD_{50} of soman in comparison with the rats pretreated with atropine alone (Fig. 1).

Based on the initial dosage and time regimen of memantine reported by Gupta and Dettbarn (1992), where soman was administered 60 min

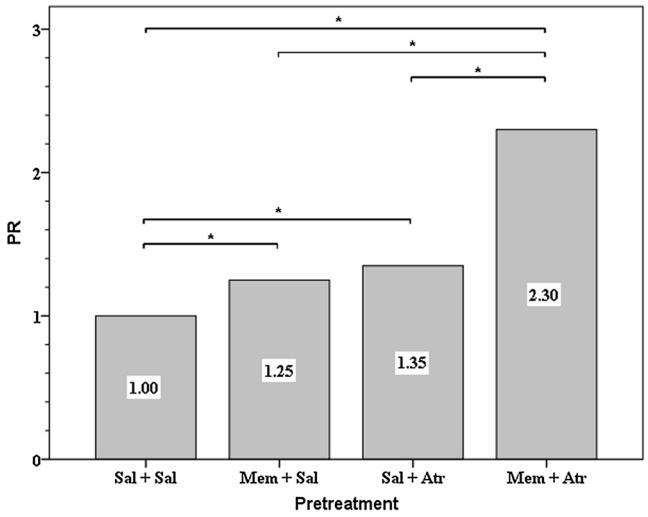

Fig. 1. Protective ratios (PRs) in rats pretreated with memantine (Mem), atropine (Atr) or their combination and poisoned with soman. Mem (18 mg/kg *sc*) or saline (Sal, 1 ml/kg *sc*) were injected 60 min, while Atr (16 mg/kg *sc*) or Sal (1 ml/kg *sc*) were administered 15 min before *sc* injection of soman. PRs were calculated based on the LD_{50} in pretreated and non-pretreated animals and their 24-hour survival. *p < 0.05.

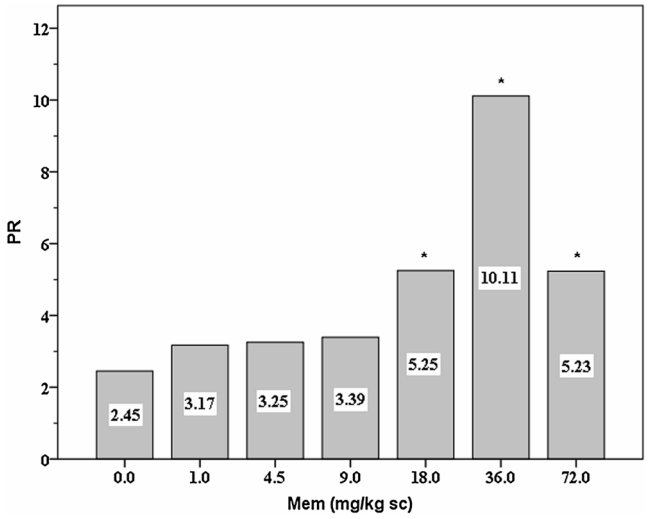

Fig. 2. Dose-dependency of the prophylactic activity of memantine (Mem) in rats poisoned with soman and treated with atropine (Atr) and HI-6. Atr (10 mg/kg) and HI-6 (50 mg/kg) were injected *im*, within 15 s after *sc* administration of soman. Protective ratios (PRs) were calculated based on the LD_{50} in treated and non-treated animals and their 24-hour survival. *p < 0.05 vs PR of Mem 0.

after memantine 18 mg/kg *im*, the pretreatment interval was first fixed at 60 min, while the doses applied were 1, 4.5, 9, 18, 36 and 72 mg/kg *sc*. All the rats received also atropine 10 mg/kg *im* and HI-6 50 mg/kg *im* within 15 s after soman and this regimen without any pretreatment assured the PR of 2.45. All the memantine doses increased the LD_{50} of soman, but only the three highest one produced significantly higher PRs of 5.25, 10.11 and 5.23 (Fig. 2).

The highest two doses of memantine – 36 and 72 mg/kg induced very serious adverse effects: motor hyperactivity and piloerection (since 5th min), ataxia and occasional abduction of rear legs (since 10th min), tremor (since 15th min), purposeless chewing movements and scratching of the nose with anterior paws (since 20th min), partial clonic spasms of the extremities (after 25th min, only after the dose of memantine of 72 mg/kg). This is why the dose of memantine of 18 mg/kg sc was considered behaviourally safe and used in further

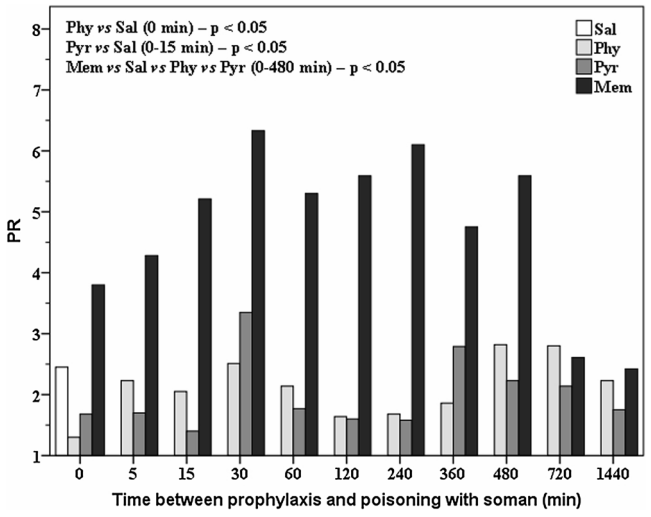

Fig. 3. Time-dependency of prophylactic efficacy of memantine (Mem), physostigmine (Phy) and pyridostigmine (Pyr) in rats poisoned with soman and treated with atropine (Atr) and HI-6. Mem (18 mg/kg *sc*), Phy or Pyr (each at a dose of 0.1 mg/kg *im*) were administered 0–1440 min before *sc* poisoning with soman. Atr (10 mg/kg *im*) and HI-6 (50 mg/kg *im*) were injected within 15 s after poisoning with soman. Protective ratios (PRs) were calculated based on the LD_{50} in treated and non-treated animals and their 24-hour survival.

experiments.

In the next experiment, the optimal dose of 18 mg/kg was fixed, while the pretreatment interval was changed. Memantine was administered concomitantly with soman (t = 0 min) or 5, 15, 30, 60 min or 2, 4, 6, 8, 12, 24 h before soman and the treatment, injected within 15 s after soman, consisting of atropine 10 mg/kg *im* and H-6 50 mg/kg *im* (Fig. 3).

Memantine pretreatment assured significantly higher PRs in all the tested time intervals ending with 8 h. The PRs ranged from 3.8 at time 0 min to 6.33 after 30 min. In same time intervals pretreatment with physostigmine or pyridostigmine (each in the dose of 0.1 mg/kg *im*) failed to increase significantly the PR obtained by atropine and HI-6, although the numerically highest PRs with these two carbamates were obtained when they were given 30 min before soman. On the contrary, physostigmine, administered at time interval 0 min and pyridostigmine, injected 0, 5 or 15 min before soman, produced PRs significantly lower that in unpretreated rats protected with atropine and HI-6 only (Fig. 3).

In further experiments the effect of pretreatments with memantine (18 mg/kg *sc* 60 min before soman) or physostigmine or pyridostigmine (both 0.1 mg/kg *im* 30 min before soman), alone or in dual combinations, were tested in saline, atropine, HI-6 or atropine and HI-6 treated rats (Fig. 4).

In rats that received saline instead of atropine and HI-6 after soman, significantly increased PRs were obtained after memantine, physostigmine, memantine plus physostigmine and memantine plus pyridostigmine, but not after pyridostigmine alone or its combination with physostigmine. In animals treated after soman with atropine, higher PRs were obtained with all prophylactic regimens, except with memantine alone. At the same time, the highest PRs, above 4, were obtained with memantine combinations with physostigmine or pyridostigmine. In HI-6 treated rats none of the three prophylactic regimens assured additional protection, but all three dual pretreatments did. When animals were treated after soman with both atropine and HI-6 (PR 2.45), memantine pretreatment assured significantly higher PR of 5.25. Similar PR was obtained with memantine plus physostigmine and especially after memantine plus pyridostigmine (PR 7.79). When administered simultaneously, physostigmine and pyridostigmine did not produce better protection than when given alone (Fig. 4).

In the next experiment the effect of memantine pretreatment

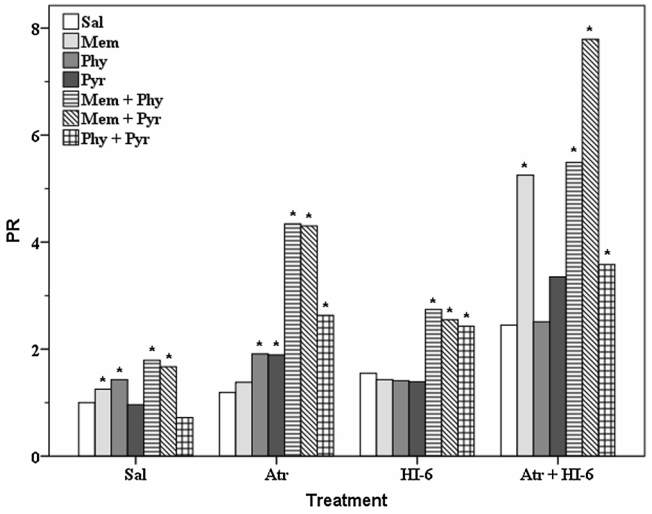

Fig. 4. Effects of treatment with atropine, HI-6 and their combination on prophylactic efficacy of memantine (Mem), physostigmine (Phy), pyridostigmine (Pyr) and their dual combinations in rats poisoned with soman. Mem (18 mg/kg *sc*) was injected 60 min, while Phy or Pyr were administered 0.1 mg/kg *im* 30 min before *sc* poisoning with soman. Atropine (10 mg/kg *im*) and HI-6 (50 mg/kg *im*) were injected within 15 s after poisoning with soman. Protective ratios (PRs) were calculated based on the LD_{50} in treated and non-treated animals and their 24-hour survival. *p < 0.05 vs soman.

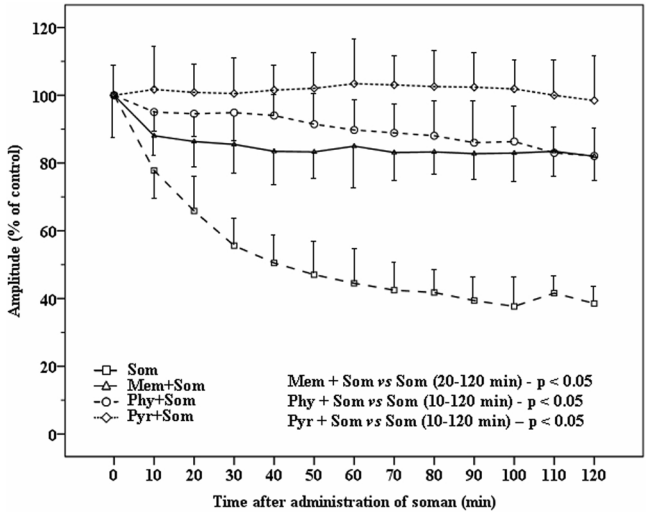

Fig. 6. Effects of soman (Som) and pretreatment with memantine (Mem), physostigmine (Phy) and pyridostigmine (Pyr) on amplitudes of contraction of phrenic nerve-diaphragm *in situ* preparation in rats poisoned with soman. Mem (18 mg/kg *sc*) was injected 60 min, while Phy or Pyr were administered 0.1 mg/kg *im* 30 min before sc injection of 0.75 LD_{50} of soman. Every marking represents mean value of six experiments, expressed as the percentage of amplitude of diaphragm contractions at the moment of soman administration. Error bars represent standard errors of mean (SEM).

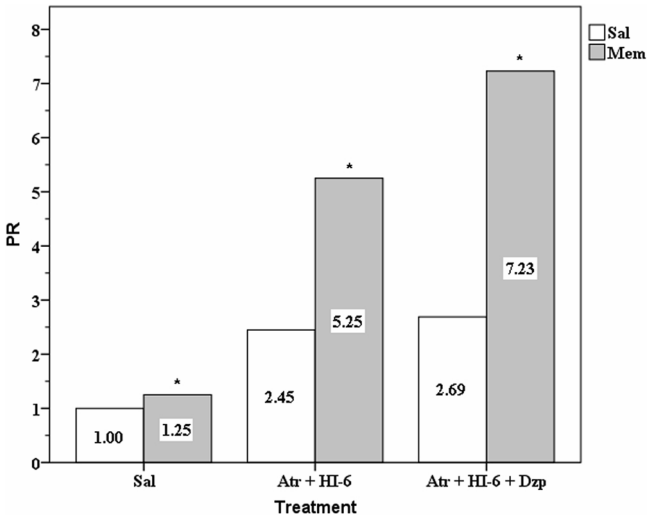

Fig. 5. Effect of treatment with diazepam on prophylactic efficacy of memantine in rats poisoned with soman. Memantine (Mem, 18 mg/kg *sc*) or saline (Sal, 1 ml/kg *sc*) were injected 60 min before soman and atropine (Atr, 10 mg/kg), HI-6 (50 mg/kg), diazepam (Dzp, 2.5 mg/kg) or saline (Sal, 1 ml/kg *sc*) were administered *im* within 15 s after *sc* poisoning with soman. Protective ratios (PRs) were calculated based on the LD_{50} in pretreated and non-pretreated animals and their 24-hour survival. *p < 0.05 vs saline control.

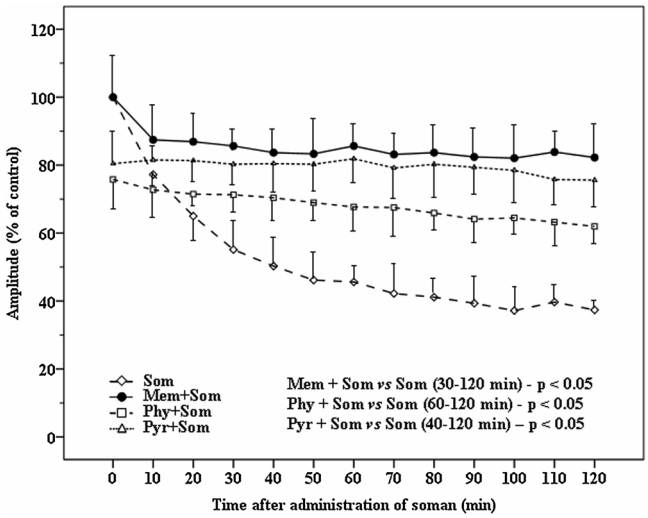

Fig. 7. Effects of pretreatment with memantine (Mem), physostigmine (Phy) and pyridostigmine (Pyr) on amplitudes of contraction of phrenic nerve-diaphragm *in situ* preparation in rats poisoned with soman (Som). Mem (18 mg/kg *sc*) was injected 60 min, while Phy or Pyr were administered 0.1 mg/kg *im* 30 min before *sc* injection of 0.75 LD_{50} of soman. Every marking represents mean value of six experiments, expressed as the percentage of amplitude of diaphragm contractions at the beginning of the experiment. Error bars represent standard errors of mean (SEM).

(18 mg/kg *sc* 60 min before soman) in rats treated with saline, atropine (10 mg/kg *im*) plus HI-6 (50 mg/kg *im*) or with atropine plus HI-6 plus diazepam (2.5 mg/kg *im*) was studied (Fig. 5).

The PR of the atropine/HI-6 combination was not significantly increased by the addition of diazepam in saline-pretreated rats (2.45 vs 2.69). At the same time, memantine prophylaxis assured significantly better protection than in saline-pretreated animals treated with saline (PRs 1.00 vs 1.25), atropine/HI-6 (2.45 vs 5.25) or atropine/HI-6/diazepam (2.69 vs 7.23). It means that memantine pretreatment assured potentiation of the atropine/HI-6 and atropine/HI-6/diazepam PRs by 2.14- and 2.69-fold, respectively. Although poisoned with the double dose of soman, the memantine-pretreated rats had significantly less severe muscle fasciculations that the saline-pretreated animals.

The effect of the three prophylactic regimens on the contractility of the diaphragm *in situ* of rats poisoned with high sublethal dose of soman (0.75 LD_{50} *sc*) was studied in two ways (Figs. 6 and 7). In Fig. 6 the basis for comparisons were amplitudes of contractions at the moment of administration of soman, while in Fig. 7 there were the amplitudes at the moment of administration of prophylactic regimens. The difference is in the fact that memantine *per se* does not affect the contractility of the rat diaphragm in situ, while both the carbamates decrease it – after 30 min by 24% and 20% in case of physostigmine and pyridostigmine,

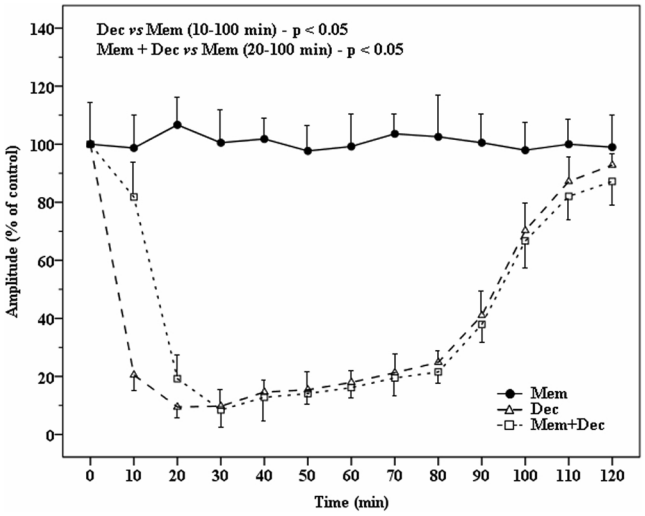

Fig. 8. Amplitudes of contraction of the phrenic nerve-diaphragm *in situ* preparation after pretreatment with memantine (Mem) and treatment with decamethonium (Dec). Mem was injected 18 mg/kg *sc* 60 min before Dec 10 mg/kg *sc*. Contractions were registered immediately before and after administration of Dec.

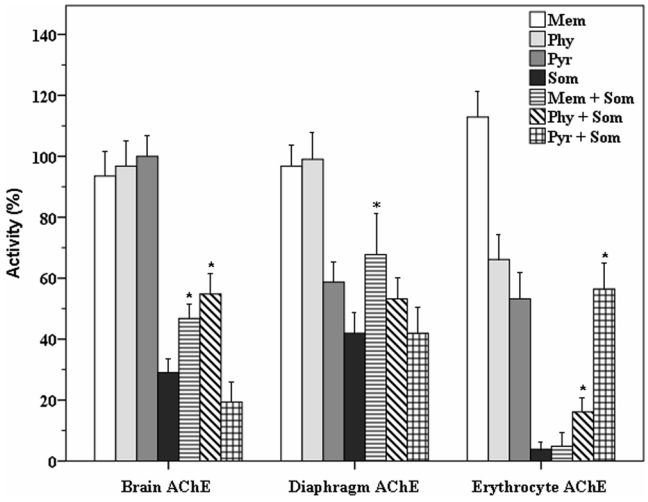

Fig. 9. Activity of acetylcholinesterase (AChE) in tissues after pretreatment of rats with memantine (Mem), physostigmine (Phy) or pyridostigmine (Pyr), and poisoning with soman (Som). Mem was injected 18 mg/kg *sc*, 60 min before soman and Phy and Pyr each 0.1 mg/kg *im*, 30 min before Som 0.75 LD_{50} *sc*. Animals were sacrificed for AChE activity determination before administration of prophylactic drugs, immediately before Som (i.e. 60 min after Mem and 30 min after Phy or Pyr) and 60 min after Som.

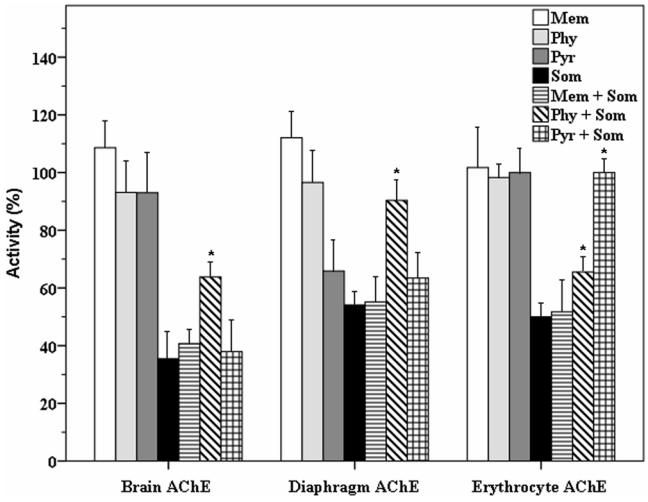

Fig. 10. Activity of acetylcholinesterase (AChE) in tissues after pretreatment of rats with memantine (Mem), physostigmine (Phy) or pyridostigmine (Pyr), poisoning with soman (Som) and treatment with HI-6. Mem was injected 18 mg/kg *sc*, 60 min before Som and Phy and Pyr each 0.1 mg/kg *im*, 30 min before Som 0.75 LD_{50} *sc*. HI-6 50 mg/kg *im* was administered within 15 s after Som. Animals were sacrificed for AChE activity determination before administration of prophylactic drugs, immediately before Som (i.e. 60 min after Mem and 30 min after Phy or Pyr and 60 min after Som.

respectively.

Starting 10 min after the administration of soman, the amplitudes become significantly lower and remain so until the end of experiment, reaching 38% of the initial value at 120 min. After memantine pretreatment, it mildly decreases, reaching significance 20 min after soman administration. Pyridostigmine assures better protection than physostigmine and memantine, reaching at 120 min after soman administration 95%, 82% and 82%, respectively (Fig. 6).

When using for comparison the initial amplitude before administration of antidotes, memantine assures best neuromuscular protection versus soman alone that becomes significant after 30 min, with this interval being 40 and 60 min in case of pyridostigmine and physostigmine, respectively. At 120 min the remaining amplitude after memantine, pyridostigmine and physostigmine is 82%, 75% and 63%, in comparison with 38% in animals treated with soman alone (Fig. 7).

Decamethonium, a depolarizing neuromuscular blocker, injected in a dose of 10 mg/kg *sc*, induced a sharp decrease in the amplitudes of contraction in the rat phrenic nerve – diaphragm preparation, reaching 21% of the control amplitude in 10 min and only 10% after 20 min. This effect started to wane after 30th min, reaching 40% of the initial amplitude after 90 min and ceasing to differ significantly from the control values after 110 min (Fig. 8).

In rats pretreated with memantine 18 mg/kg *sc* 60 min before decamethonium the depolarizing block occurred more slowly – the amplitudes being 82% and 20% after 10 and 20 min, respectively. After that, the amplitudes did not differ from those ones in the unpretreated animals.

Fig. 9 contains activities of AChE in brain, diaphragm and erythrocytes 120 min after memantine (18 mg/kg *sc*), 90 min after physostigmine or pyridostigmine (each 0.1 mg/kg *im*) and 60 min after soman 0.75 LD50 *sc*, without and with memantine or carbamate pretreatment. Soman induced inhibition of AChEs by 70%, 57% and 95% in brain, diaphragm and erythrocytes, respectively. Memantine *per se* did not influence the activity of the enzymes, while physostigmine and pyridostigmine *per se* inhibited AChE in erythrocytes by 32% and 55%, respectively and only pyridostigmine in the diaphragm (by 43%).

Both centrally acting prophylactic drugs – memantine and physostigmine – significantly increased the brain AChE activity in soman-poisoned rats, from 30% to 47% and 56%, respectively. In the diaphragm, memantine also increased its AChE activity from 43% to 69%. Memantine pretreatment did not protect the erythrocyte AChE from inhibition by soman, but physostigmine and pyridostigmine assured increase in its activity from 5% to 17% and 57%, respectively (Fig. 9).

The corresponding results, but obtained in rats treated with HI-6 50 mg/kg *im* within 15 s after soman are shown in Fig. 10. The oxime *per se* had no effect on brain AChE activity 60 min after soman, but it did increase the AChE activity in the diaphragm and especially in the erythrocytes. Addition of HI-6 practically annulled all the memantine-induced increase in the AChE activity. Oxime HI-6 did not offset the protective effect of physostigmine on brain AChE; it increased its activity from 37% to 65%. Oxime HI-6 potentiated the protective effect of physostigmine and pyridostigmine on AChE in diaphragm and erythrocytes, by increasing its activity from 55% to 92% and 64% and from 50% to 65% and 101%, respectively (Fig. 10).

Table 2
Median lethal, effective and toxic doses of memantine physostigmine and pyridostigmine in rats.

Parameter	Unit	Memantine HCl (*sc*)	Physostigmine sulphate (*im*)	Pyridostigmine bromide (*im*)
Molecular wt.		217.8	648.8	261.2
LD_{50}	mg/kg	147.32	1.49	3.33
	μmol/kg	676.40	2.30	12.75
ED	mg/kg	18	0.1	0.1
	μmol/kg	82.65	0.15	0.3
TD_{50}	mg/kg	4.13	0.22	0.89
	μmol/kg	18.69	0.33	3.41

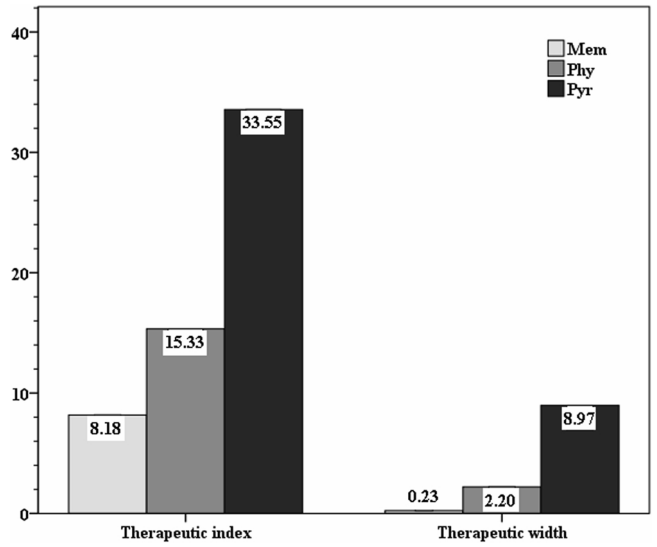

Fig. 11. Therapeutic indices and therapeutic widths of memantine, physostigmine and pyridostigmine in rats. Therapeutic index is a ratio between the LD_{50} and the prophylactic dose, while therapeutics width is a ratio between TD_{50} (ED_{50} for rotarod) and the prophylactic dose, all in micromoles.

Lethal, effective and toxic effect of the three prophylactic drugs are shown in Table 2, where the ED_{50} for the rotarod test was considered as toxic dose 50% (TD_{50}).

Based on micromoles, physostigmine appears to have a lethal potential 5.54-fold higher than pyridostigmine and 294.08-fold higher than memantine. At the same time, the effective prophylactic dose of memantine is highest – 551 and 275.5 times higher than physostigmine and pyridostigmine, respectively. Physostigmine is potentially the most toxic substance of the three, 33 times more toxic than pyridostigmine and 57.46 times more toxic than memantine.

Fig. 11 contains data on the therapeutic indices and therapeutic widths of the three prophylactic drugs.

Based on the data in Fig. 11, it can be seen that memantine has the lowest therapeutic index, almost half of the one for physostigmine and almost a quarter of the one for pyridostigmine. In other words, the effective dose of memantine is 12.22% of its LD_{50}, in comparison with only 6.52% and 2.98% of the respective LD_{50} values for physostigmine and pyridostigmine. These differences are even higher when it comes to therapeutic width, which amounts only 0.23 for memantine, in comparison with 2.2 and 8.97 for physostigmine and pyridostigmine, respectively. The behaviourally toxic dose TD_{50}, expressed as the ED_{50} value at the rotarod test, makes only 2.8% of its LD_{50} and 14.34% and 26.75% of the corresponding LD_{50}s for physostigmine and pyridostigmine. It means that even the small fractions of memantine's lethal dose impair behavior in rats.

4. Discussion

4.1. General potential of memantine prophylaxis in rats poisoned with soman

The results presented in this paper clearly show that memantine exerts better protection from poisoning with soman in rats than the standard carbamate prophylactic regimens and even more so in animals that were treated with atropine and HI-6 within 15 s after poisoning with soman. In this context memantine alone and in combination with pyridostigmine assured PRs of 5.25 and 7.79, respectively.

These PRs are significant, especially after having in mind that the therapeutic efficacy of standard antidotes against soman poisoning is significantly lower in rats than, say, guinea pigs (Berry et al., 1971; Gordon et al., 1978; Lennox et al., 1985; Dawson, 1994). Besides, these PRs are significantly higher than 3.5, a PR obtained in physostigmine-pretreated (0.07 mg/kg *im*, 30 min before soman) male rats poisoned with soman *sc* and treated with atropine (80 mg/kg *sc*), oxime HI-6 (25 mg/kg *im*) and diazepam (5 mg/kg *im*) (Sket, 1993). It is more plausible to compare this Sket's result with our own, obtained with memantine pretreatment and triple antidotal treatment regimen with atropine (10 mg/kg *im*), HI-6 (50 mg/kg *im*) and diazepam (2.5 mg/kg *im*), where a PR above 7 was obtained, which is at least two times higher than PRs obtained with physostigmine pretreatment and atropine/oxime/diazepam treatment, where the PRs were within the range of 1.8–2.3 (Harris et al., 1984), 2 (Lennox et al., 1985) and 3.5 (Sket, 1993). Although our dose of HI-6 was twice as big as the Sket's (50 vs 25 mg/kg), the doses of atropine and diazepam used in the present study were 8 and 2 times lower, respectively.

Moreover, memantine *per se*, like physostigmine (Inns and Marrs, 1992) and unlike pyridostigmine, assured small, but significant protection against soman. Both the adamantanes (Majerski et al., 1976) and physostigmine (Stojiljković et al., 1989) are highly lipophilic and gain access to the central nervous system (Wesemann et al., 1982, 1983; Somani and Khalique, 1986; King and Somani, 1987; Somani, 1989), which is not the case with pyridostigmine (Birtley et al., 1966). This is why this small protection with memantine and physostigmine when administered alone can counteract toxic effects of soman that are primarily of central origin (Wolthuis et al., 1981; Misulis et al., 1987; Škrbić et al., 2017). The PRs for memantine and physostigmine registered in the present study – 1.25 and 1.45, respectively, are close to the PR of 2.1 obtained after pretreatment with physostigmine 0.07 mg/kg *im* (Sket, 1993), but are much lower than 3.5, obtained in rats poisoned with soman *sc* 15 min after the *iv* administration of ten times larger dose of physostigmine (1 mg/kg) and atropine (10 mg/kg) (Fleisher and Harris, 1965). The reason for such a high PR was a large dose of physostigmine that reversibly inhibited (and thus protected from soman inhibition) a larger percentage of brain AChE than the one inhibited by 10 times smaller dose of physostigmine (0.1 mg/kg *im*) in the present study. The problem of high *per se* toxicity of such a large dose of physostigmine was solved by co-administration of otherwise therapeutic dose of atropine (10 mg/kg *im*) that not only antagonised the central muscarinic toxic effects of physostigmine, but of soman, as well.

From yet another study it can be seen that the obtained PR in rats poisoned with soman is proportional to the percentage of the full blood AChE inhibited by physostigmine or pyridostigmine pretreatment, with PRs corresponding to 70%-inhibition of AChE being 2.1 and 2.4, respectively (Lennox et al., 1985). Like in Fleisher and Harris' paper, here too the tolerability of these two carbamate pretreatment regimens was assured by co-administration of the antimuscarinic drug atropine (16 mg/kg *im*) and antinicotinic drug mecamylamine (0.8 mg/kg *im*) which were injected 1 min after soman. In the present study, pretreatment with same carbamates and treatment with atropine 10 mg/kg *im* assured PRs of around 2. This is in accordance with the PR of 2, obtained in rats pretreated with physostigmine 0.1 mg/kg *im* and atropine 17.4 mg/kg *im*, both 10 min before soman (Berry et al., 1971) and the

PR of 1.7 obtained with pyridostigmine (0.075 mg/kg *im*) pretreatment 20 min before soman and treatment with atropine 17.4 mg/kg *im* and oxime P2S 30 mg/kg *im* (Gordon et al., 1978).

Addition of atropine (PR 1.35) to memantine (PR 1.25) prophylaxis in our study also resulted in the additive synergism (PR 2.3). This conclusion is in accordance with the finding that in rats sublethally intoxicated with soman atropine and memantine effectively antagonised muscarinic (hypersalivation, lacrymation) and nicotinic toxic phenomena (tremor, muscle fasciculations, convulsions) (Gupta et al., 1987; Gupta and Dettbarn, 1992).

It is important to point out that the memantine prophylactic combinations with physostigmine or pyridostigmine in rats treated after soman with atropine achieved higher PRs of 4.4 and 4.3, respectively, than the dual carbamate regimen, with PR of 2.6, which was not significantly different from PRs of physostigmine or pyridostigmine when administered as monocomponent prophylactic regimens. In a similar experiment in guinea pigs pretreated also with scopolamine 0.8 mg/kg *im*, prophylactic combination of physostigmine and pyridostigmine indeed could not produce higher protection than physostigmine, while pyridostigmine was inferior (Solana et al., 1990b). This was explained by the known tropism of soman for the brain structures, where a compound with quaternary nitrogen like pyridostigmine cannot gain access to (Solana et al., 1990b).

In the present experiment, oxime HI-6, administered alone in a dose of 50 mg/kg *im*, assured PR against soman poisoning of almost 1.6, which was significant. There are no data in the literature that this dose of HI-6 *per se* can protect rats against soman, because of which oximes, including HI-6, are administered along with atropine or with some other anticholinergic (Clement, 1981; Clement and Lockwood, 1982). There is only one report that HI-6 monotherapy could exert a significant protection against soman (PR 2.5), but the dose of HI-6 was much higher, 125 mg/kg *ip* (Shih et al., 1991b). The reason for such a low antidotal efficacy of oximes, including HI-6 that reactivates AChE inhibited by soman (Oldiges and Schoene, 1970) is their weak penetration into the mammalian central nervous system (Pantelić and Maksimović, 1982; Ligtenstein and Kossen, 1983; Sket and Brzin, 1986) and especially into the pontomedullary region of the brain, where respiratory and cardiovascular centres are located and where soman exerts most of its toxic effects (Ligtenstein et al., 1988).

Prophylaxis with memantine or physostigmine or pyridostigmine did not increase this protection of HI-6 treatment significantly, but all three dual prophylactic combinations did so, producing PRs of 2.5-2.8. The explanation would be a better coverage of central and peripheral signs of soman toxicity provided by dual prophylactic regimens. In support of this stand the results of the experiment where pretreatment of guinea pigs with pyridostigmine and its tertiary (i.e. lipophilic) analogue 3-(N,*N*-dimethylcaramyloxy)-1-methyl-Δ^3-tetrahydropyridine (THP), without any other therapy, protected 60% of animals from dying following a challenge with 2 LD_{50} of soman (Ray et al., 1991). Although this dual prophylactic regimen did not protect guinea pigs from the occurrence of soman-induced convulsions, it significantly shortened the recovery time from 24 h (in unprotected animals and those ones protected with pyridostigmine or THP monotherapies) to only 1.6 h (Ray et al., 1991).

4.2. Dose-dependency of memantine protection

In rats treated after soman with atropine and HI-6, memantine assured a clear dose-dependent protection. The lowest dose that significantly exceeded the protection of the atropine/HI-6 combination (PR 2.45) was 18 mg/kg (PR 5.25). The PRs of the yet higher doses of memantine – 36 and 72 mg/kg – were around 10 and 5, respectively. The reason why the highest dose of memantine did not produce even better protection is in the fact that this dose was obviously toxic, causing convulsions in some animals. These results are in accordance with Gupta's findings based on the studying of memantine dose range of 5–50 mg/kg, where doses of memantine higher than 20 mg/kg induced serious behavioural impairments, including hyperexcitability, stereotypic movements and convulsions (Gupta and Kadel, 1989, 1990, 1991a, 1991b, 1991c).

4.3. Time-dependency of memantine and carbamate protection

The chosen prophylactic dose of memantine (18 mg/kg) exerts a long-lasting protection, until 8 h after its *sc* administration. During that period, in rats treated with atropine and HI-6 memantine assures PRs that vary from 3.8 (at time 0, i.e. when it is administered within 15 s after soman) to 6.33 (30 min before soman), which is significantly higher than PR of 2.45 in unpretreated animals. At the same time, neither of the two carbamates assured any additional protection against soman, due to the species-dependent resistance of rats to carbamate pretreatment before soman intoxication (Gordon et al., 1978; Somani and Dube, 1989). Moreover, both carbamates at time 0 and pyridostigmine at times 5 and 15 min, even potentiated the toxicity of soman by significantly decreasing the atropine/HI-6 protection. The reason for that is probably the summation of AChE-inhibiting potencies of carbamates and soman in peripheral tissues, since it was already shown that the inhibition greater than 65% can sensitise the animals for soman-induced convulsions and lethality (Shiloff and Clement, 1986). In addition to that, it was shown that physostigmine prophylaxis, although generally favourable in terms of final outcomes, shortens the time between soman administration and onset of signs of poisoning – from 4.27 to 1.71 min (Sket, 1993). Since this phenomenon was described 30 min after prophylaxis with physostigmine 0.07 mg/kg *im*, it is obvious why we obtained potentiation of soman toxicity in short pretreatment intervals (carbamates 0–15 min before soman). The difference in the duration of this harmful effect arises from the faster onset and longer duration of action of pyridostigmine in comparison to physostigmine.

In animals poisoned with soman physostigmine prophylaxis loses its efficacy after 1.5 h in rats (Sket, 1993) and after 1 h in guinea pigs (Berry et al., 1971). At the same time, pyridostigmine prophylaxis in guinea pigs assures good protection against soman poisoning until 4 h (Inns and Marrs, 1992) and this was the reason for abandoning the physostigmine, an agent with central effects like soman, in favour of pyridostigmine, an only peripherally, but much longer-acting agent (Marrs et al., 1996). In the present study, however, it was shown that the safety pretreatment interval for memantine was even longer – 8 h.

4.4. Mechanism of prophylactic effect of memantine against soman poisoning in rats

There are some clear indications that the mechanism of action of memantine involves AChE, although neither in the present study nor in the literature any AChE inhibition by memantine *per se* is reported (Gupta and Kadel, 1989; McLean et al., 1992). Indeed, memantine decreases the level of AChE inhibition caused by soman or other AChE inhibitors (Gupta and Dettbarn, 1992; McLean et al., 1992). In the present study such protection was demonstrated in rat brain and diaphragm, which explains the favourable neuromuscular effects of memantine. At the same time, memantine lacks the oxime functional group and is therefore incapable of nucleophilic attack on the phosphorus atom of the OPC-AChE complex, which was confirmed *in vitro*, where memantine 2×10^{-5} mol/l failed to reactivate human erythrocyte AChE inhibited by tabun, sarin, soman or VX (Bregovec et al., 1992).

Addition of HI-6 treatment annulled the biochemical protective effects of memantine on brain and diaphragm AChE. At the same time, it increased the percentage of uninhibited AChE in pyridostigmine-pretreated rats and kept the physostigmine protection significant. The reason for this discrepancy is in the fact that HI-6 increased AChE activity in the unpretreated animals, raising thus the control remaining

AChE activity. In other words, memantine biochemical effect were just masked by HI-6.

Even from the first Gupta's paper it was clear that memantine was devoid of any antimuscarinic property, since it could not antagonise in animals intoxicated with various carbamate and OP compounds, including nerve agents, classic muscarinic phenomena, such as hypersalivation and chromodacryorrhea and atropine had to be used instead (Gupta and Dettbarn, 1992). At the same time, memantine was efficient in counteracting nicotinic toxic phenomena, such as muscle fasciculations (Gupta and Kadel, 1990). Although memantine *per se* did not affect amplitude of contractions of the rat phrenic nerve-diaphragm preparation *in situ*, which puts it ahead of physostigmine and pyridostigmine, it manages to alleviate the soman-induced depolarisation block quantitatively at the same level as the two carbamates and with even faster onset.

It seems that the neuromuscular effect of memantine originates from its ability to block the nicotinic receptor-sodium ionophore complex (Masuo et al., 1986; Tsai et al., 1989), which should be beneficial for the protection of neuromuscular transmission against acetylcholine-induced depolarisation block that follows the AChE inhibition. In support of this notion, memantine equally or even more effectively than the competitive neuromuscular nicotinic receptor antagonist d-tubocurarine, protected skeletal muscles in rats poisoned with tabun, sarin, soman or VX (Gupta and Dettbarn, 1992). At the same time, memantine pretreatment could not effectively prevent the occurrence of the depolarisation block induced by decamethonium, a direct neuromuscular nicotinic receptor antagonist, although it did delay to some extent the onset of the full neuromuscular block. Although the interaction at the receptor level cannot be completely ruled out, it seems that memantine protects the neuromuscular transmission rather via its effect on AChE.

Memantine also possesses some anticonvulsive potential, which was shown in soman-induced convulsions, as well (McLean, 1987; Antonijević et al., 2011). In support of this, other drugs belonging to the class of glutamate receptor antagonists - noncompetitive NMDA receptor antagonists – dizocilpine (MK-801) (Braitman and Sparenborg, 1989; Sparenborg et al., 1992), procyclidine (Price et al., 1989), ketamine (Clinton et al., 1988; Dorandeu et al., 2005) – AMPA antagonists – NBQX (Löscher and Hönack, 1994) and kainate receptor antagonists – topiramate (Acon-Chen et al., 2016) also exert similar activity. Although ketamine fails to decrease acetylcholine level after soman in the microdialysis study in rats (Acon-Chen et al., 2016), it exerts neuroprotective effects when used along with atropine (Shih et al., 1999; Dorandeu et al., 2005; Myhrer et al., 2010). Since it is known that soman-induced convulsions seriously affect both the morphology and the functioning of CNS, anticonvulsant activity of memantine may represent its dominant antidotal mechanism. A proof of this notion is the fact that memantine pretreatment doubled the PR of atropine/HI-6 therapy, but almost tripled the PR of the therapeutic regimen where the anticonvulsant drug diazepam was included. Additional indirect proof of the concept is the notion that caramiphen, a compound with combined anticholinergic and anti-NMDA properties, stops the soman-induced seizures, decreases neuronal loss, and neuronal degeneration even when administered 30 or 60 min after soman (Figueiredo et al., 2011). Similar results were obtained by the AMPA/GluK1 receptor antagonist LY293558 and the specific GluK1 antagonist UBP302 in 21-days old rats exposed to soman (Miller et al., 2015). Microinjection into basolateral amygdala or area tempestas of dizocilpine (MK-801), another NMDA receptor antagonist, 30 min before systemic poisoning with sarin, prevented the occurrence of seizures (Skovira et al., 2010).

4.5. Behavioural tolerability and overall assessment of the prophylactic potential of memantine

All three prophylactic drugs *per se* significantly diminished the capability of rats to perform the rotarod test, with ED_{50} values 4.13, 0.22 and 0.89 mg/kg for memantine, physostigmine and pyridostigmine, respectively. If we use these values to divide the corresponding values with the prophylactic doses used in the present study – 18, 0.1 and 0.1 mg/kg, respectively, therapeutic width will be obtained, showing that physostigmine and pyridostigmine are 10 and 40 times less behaviourally toxic than memantine. Gupta and co-workers have also reported on the increase in spontaneous motoric activity induced by the same dose of 18 mg/kg of memantine (Gupta and Kadel, 1989). This result is supported by literature data, according to which memantine more intensively than amantadine potentiated dopaminergic phenomena in rodents, such as antikataleptic effect, circling behavior, stereotypies and stimulates their spontaneous motoric activity (Maj et al., 1974; Costall and Naylor, 1975; Maj, 1982; Wesemann et al., 1983). Myhrer and Aas (2016) also described significant behavioural adverse effects after use of anticonvulsants with NMDA-blocking activity.

It seems that memantine blocks the ionophore within the NMDA receptor in the striatal neurons and thus decreases the glutamate-induced release of acetylcholine (Lupp et al., 1992). Memantine affects the rotarod performance not only by central, but also by means of peripheral mechanisms. It acts as muscle relaxant by antagonising neuromuscular NMDA receptors (Schwarz et al., 1992) that exist at least during the development of the neuromuscular function (Personius et al., 2016).

In conclusion, although memantine prophylaxis assures significant and long-lasting protection against soman poisoning in rats that lasts for 8 h and outperforms carbamate prophylactic effects, its narrow therapeutic index and therapeutic width seriously limit its potential as a pretreatment agent. Further research is needed to ascertain whether the combination of lower, behaviourally non-toxic doses of memantine and pyridostigmine could offer good protection, since the combination of the usual prophylactic dose of memantine and pyridostigmine resulted in PR of almost 8. Moreover, despite its behavioural effects, memantine seems to be beneficial antidote when administered after soman, along with atropine/HI-6/diazepam therapy. Mechanism of the antidotal effect of memantine against soman poisoning appears to be a combination of AChE-protecting and NMDA receptor-blocking action.

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