Dizocilpine Improves Beneficial Effects of Cholinergic Antagonists in Anticholinesterase-Treated Mice

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Mice were administered anticholinesterase pesticides dichlorvos (DDVP) or methomyl (MET). Both DDVP and MET induced dose-dependent seizures and lethality in mice. The muscarinic antagonist atropine (ATR, 1.8 mg/kg) did not prevent seizures but diminished the lethality induced by DDVP or MET. The nicotinic antagonist mecamylamine (MEC, 1 mg/kg) affected neither DDVP-induced seizures nor DDVP- and MET-induced lethality, but diminished MET-induced seizures. At a higher dose (10 mg/kg), MEC attenuated seizures produced by MET, but not DDVP, and decreased lethality of both anticholinesterases. The N-methyl-D-aspartate (NMDA) antagonist dizocilpine (MK-801, 1 mg/kg) prevented DDVP-, but not MET-induced seizures. MK-801 did not affect DDVP- or MET-induced lethality. Concurrent administration of ATR and MK-801 prevented the occurrence of DDVP-but not MET-induced seizures. MK-801 coadministered with ATR enhanced its protective effect against DDVP- or MET-induced lethality in mice. Coinjection of MEC (at both doses studied) and MK-801 completely prevented seizures produced by both acetylcholinesterase (AChE) inhibitors. Coadministration of MEC (1 mg/kg) and MK-801 protected mice against DDVP or MET lethality. MK-801 administered along with MEC at 10 mg/kg enhanced antilethal effects of the nicotinic antagonist in DDVP- or MET-treated mice. With respect to the mechanism underlying anticholinesterase-induced neurotoxicity, muscarinic and nicotinic, as well as NMDA receptors, seem to play major roles. The results suggest that combined treatment with cholinergic and NMDA antagonists might be beneficial in anticholinesterase-induced poisonings.

Key Words: anticholinesterase; NMDA antagonist; atropine; mecamylamine; mice; seizures; lethality; nicotinic; muscarinic.

Acetylcholine (ACh), a potent excitatory neurotransmitter acting at muscarinic and nicotinic receptors, plays a major role in the central nervous system (CNS). Physiological breakdown of ACh is catalyzed by acetylcholinesterase (AChE). Inhibition of this enzyme results in an overabundance of ACh in the synaptic cleft and occurrence of neurotoxic symptoms, including convulsive seizures. Lethality is usually a consequence of respiratory failure of both peripheral and central origin (Marrs, 2000). According to their chemical structures, AChE-blocking agents may be classified into at least two groups: organophosphates (OPs) and carbamates. These compounds are widely employed as insecticides (Marrs, 2000). Among AChE inhibitors, particular attention has been paid to OP nerve gases such as sarin or soman, chemical warfare agents (Ohbu et al., 1997).

AChE inhibitor-induced poisonings continue to be an important clinical issue (Bardin et al., 1994). Acute intoxications with OPs or carbamates usually eventuate from suicidal or erroneous ingestion. Nevertheless, other sources of exposure have been reported (Cable and Doherty, 1999; Ohbu et al., 1997; Olivier et al., 1988).

The treatment of poisonings produced by AChE inhibitors has remained unchanged for many decades with the muscarinic antagonist atropine (ATR) being used as a primary antidote. In OP intoxications, AChE reactivators (oximes) are used in addition to ATR. If seizures occur during the course of poisoning, benzodiazepine anticonvulsants are applied (Holsteg et al., 1997). This treatment regimen has, however, several drawbacks. Very high doses of ATR administered in AChE inhibitor-induced intoxications may cause serious adverse effects (Bowden and Krenzelok, 1997). Moreover, ATR counteracts only the muscarinic effects of the anticholinesterases (Bardin et al., 1994). Numerous researchers have called into question the relevance of oxime treatment. None of the available oximes can be regarded as a universally suitable AChE reactivator, and identification of the exact cause of poisoning is often impossible. Another aspect undermining the clinical value of the oximes is the aging of the enzyme-inhibitor complex, limiting the potential for AChE reactivation (Worek et al., 1997). Moreover, the ability of oximes to cross the blood-brain barrier and reactivate AChE in the CNS is negligible. Various authors have demonstrated the toxicity of oximes (Dawson, 1994; Marrs, 2000). Finally, benzodiazepines commonly administered to control AChE inhibitor-related seizures may occasionally depress respiratory and cir-
culatory centers in the brainstem. Thus, benzodiazepines may potentiate the OP- or carbamate-induced depression of these centers (Munro et al., 1990).

It is commonly accepted that many symptoms of AChE inhibitor-induced intoxications result from stimulation of both muscarinic and nicotinic receptors by ACh. Notwithstanding, studies on the involvement of nicotinic receptors in poisonings induced by AChE-inhibiting compounds are relatively few. They suggest, however, that the effects of anticholinesterases may not only eventuate from excessive stimulation of nicotinic receptors by ACh, but also from their direct effects on nicotinic receptors (Bakry et al., 1988). The influence of nicotinic antagonists on seizures induced by OP or carbamate insecticides has not been studied.

Continuous risk of exposure to anticholinesterases, doubts concerning the available treatment and uncertainty about the mechanisms underlying the neurotoxicity of OPs, and carbamates has increased researchers’ interest in this topic. Of numerous receptor systems implicated in the lethal and convulsant effects of AChE inhibitors, the excitatory amino acid (EAA) system has gained the most interest (Shih and McDonough, 1997). EAs, endogenous neurotransmitters, and neuromodulators in the CNS have been implicated in neurodegenerative and epileptogenic processes and EAA antagonists exert neuroprotective and anticonvulsive effects in various experimental models. It has been shown that in soman-induced seizures and death, ACh receptors are closely interrelated with EAA systems (Shih and McDonough, 1997). Recently, EAA antagonists coadministered with ATR were shown to counteract the lethality and seizures produced by OP or carbamate pesticides (Dekundy et al., 2001). These reports encourage the further investigation of reciprocal relationships between cholinergic and EAA systems. Thus, in the present study, we examined the investigation of reciprocal relationships between cholinergic systems (Munro et al., 1990). Ethical Committee in Lublin, Poland.

Materials and Methods

Animals. Male Swiss mice weighing 20–26 g (HZL, Warsaw, Poland) were used in the experiment. The animals were housed in a room with controlled temperature (21–22°C), light (12-h light-dark cycle), and humidity (50 ± 10%). The animals were allowed free access to standard laboratory food (LSM, Agropol, Motycz, Poland) and tap water. Assignment of subjects to experimental groups (n = 8–10) was randomized. The tests were performed between 0900 and 1300 h. The experiments were performed according to the Guiding Principles in the Use of Animals in Toxicology and approved by the Ethical Committee in Lublin, Poland.

Chemicals

Sources. DDVP (2,2-chlorovinyl dimethyl phosphate) and MET (S-methyl-N-(methylcarbamoyloxy)thio-acetimidate) were obtained from Sigma Chemical Co. (St. Louis, MO). MK-801 (dizocilpine maleate) and MEC (mecamylamine hydrochloride) were obtained from Research Biochemicals International (Natick, MA). (–) Nicotine (base form) was purchased from Sigma Chemical Co. (St. Louis, MO). ATR (atropine sulfate) was purchased from Sigma Chemical Co. (St. Louis, MO). ATR (atropine sulfate) was purchased from Sigma Chemical Co. (St. Louis, MO). ATR (atropine sulfate) was purchased from Sigma Chemical Co. (St. Louis, MO). ATR (atropine sulfate) was purchased from Sigma Chemical Co. (St. Louis, MO). ATR (atropine sulfate) was purchased from Sigma Chemical Co. (St. Louis, MO). ATR (atropine sulfate) was purchased from Sigma Chemical Co. (St. Louis, MO). ATR (atropine sulfate) was purchased from Sigma Chemical Co. (St. Louis, MO). ATR (atropine sulfate) was purchased from Sigma Chemical Co. (St. Louis, MO). ATR (atropine sulfate) was purchased from Sigma Chemical Co. (St. Louis, MO).

Preparation of solutions. DDVP was mixed with powdered acacia and distilled water in a dry mortar in the proportions of 2:1:1.5 (weight/weight/volume), respectively. Afterwards saline was added to obtain desired concentrations. MET, ATR, MEC, MK-801, and nicotine were dissolved in saline.

Administration. DDVP and MET were administered ip at the minimum of three doses producing lethal or convulsive effects between 0 and 100%, in order to further analyze dose-effect curves. ATR was administered sc at the dose of 1.8 mg/kg (effective against both OP and carbamate-induced lethality; Dekundy et al., 2001), 30 min before either DDVP or MET. MEC was administered ip at doses of 1 and 10 mg/kg, 30 min prior to pesticides studied. The MEC dose was determined as follows. The CD90 value (the dose inducing convulsive seizures in 97% of animals) was established for the nicotinic agonist, nicotine. This dose was 13 mg/kg. Then a MEC ED90 value (dose protecting 50% of animals from the occurrence of seizures) was determined in mice treated with nicotine at its CD90. The ED90 for MEC was 0.5 mg/kg. A dose two-fold greater than the ED90 was used in further experiments. MEC was also administered at the dose of 10 mg/kg, which has proven to exert central pharmacological effects in vivo (Jones and Shannon, 2000). MK-801 was administered ip at the dose of 1 mg/kg, 30 min before DDVP or MET. This dose was previously reported to be effective against anticholinesterase-induced seizures and lethality (Braitman and Sparenborg, 1989; Sparenborg et al., 1992). Dosages of all drugs were based on the weight of their salt forms. ATR was administered ip at the dose of 1 mg/kg, 30 min before DDVP or MET. This dose was previously reported to be effective against anticholinesterase-induced seizures and lethality (Braitman and Sparenborg, 1989; Sparenborg et al., 1992). Dosages of all drugs were based on the weight of their salt forms. ATR was administered ip at the dose of 1 mg/kg, 30 min before DDVP or MET. This dose was previously reported to be effective against anticholinesterase-induced seizures and lethality (Braitman and Sparenborg, 1989; Sparenborg et al., 1992). Dosages of all drugs were based on the weight of their salt forms. ATR was administered ip at the dose of 1 mg/kg, 30 min before DDVP or MET. This dose was previously reported to be effective against anticholinesterase-induced seizures and lethality (Braitman and Sparenborg, 1989; Sparenborg et al., 1992). Dosages of all drugs were based on the weight of their salt forms. ATR was administered ip at the dose of 1 mg/kg, 30 min before DDVP or MET. This dose was previously reported to be effective against anticholinesterase-induced seizures and lethality (Braitman and Sparenborg, 1989; Sparenborg et al., 1992). Dosages of all drugs were based on the weight of their salt forms. ATR was administered ip at the dose of 1 mg/kg, 30 min before DDVP or MET. This dose was previously reported to be effective against anticholinesterase-induced seizures and lethality (Braitman and Sparenborg, 1989; Sparenborg et al., 1992). Dosages of all drugs were based on the weight of their salt forms. ATR was administered ip at the dose of 1 mg/kg, 30 min before DDVP or MET. This dose was previously reported to be effective against anticholinesterase-induced seizures and lethality (Braitman and Sparenborg, 1989; Sparenborg et al., 1992). Dosages of all drugs were based on the weight of their salt forms. ATR was administered ip at the dose of 1 mg/kg, 30 min before DDVP or MET. This dose was previously reported to be effective against anticholinesterase-induced seizures and lethality (Braitman and Sparenborg, 1989; Sparenborg et al., 1992). Dosages of all drugs were based on the weight of their salt forms. ATR was administered ip at the dose of 1 mg/kg, 30 min before DDVP or MET. This dose was previously reported to be effective against anticholinesterase-induced seizures and lethality (Braitman and Sparenborg, 1989; Sparenborg et al., 1992). Dosages of all drugs were based on the weight of their salt forms. ATR was administered ip at the dose of 1 mg/kg, 30 min before DDVP or MET. This dose was previously reported to be effective against anticholinesterase-induced seizures and lethality (Braitman and Sparenborg, 1989; Sparenborg et al., 1992). Dosages of all drugs were based on the weight of their salt forms. ATR was administered ip at the dose of 1 mg/kg, 30 min before DDVP or MET. This dose was previously reported to be effective against anticholinesterase-induced seizures and lethality (Braitman and Sparenborg, 1989; Sparenborg et al., 1992). Dosages of all drugs were based on the weight of their salt forms. ATR was administered ip at the dose of 1 mg/kg, 30 min before DDVP or MET. This dose was previously reported to be effective against anticholinesterase-induced seizures and lethality (Braitman and Sparenborg, 1989; Sparenborg et al., 1992).

RESULTS

Seizure Activity

DDVP- or MET-induced seizures. Both DDVP and MET administration resulted in dose-dependent behavioral seizures. The CD90 values for DDVP and MET determined after 2 h of
Behavioral seizures were induced by dichlorvos (DDVP) in mice pretreated with a muscarinic antagonist atropine (ATR, 1 mg/kg) or a nicotinic antagonist mecamylamine (MEC1, 1 mg/kg; MEC10, 10 mg/kg) coadministered with a glutamate antagonist dizocilpine (MK-801, 1 mg/kg). CD₉₀ values (expressed in mg/kg) were determined and compared using probit analysis (Litchfield and Wilcoxon, 1949). NA, not applicable; NS, not statistically significant.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Treatment</th>
<th>DDVP CD₉₀ (95% confidence limit)</th>
<th>p &lt; 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>DDVP</td>
<td>19.7 (16.7–23.3)</td>
<td>NA</td>
</tr>
<tr>
<td>B</td>
<td>DDVP + ATR</td>
<td>20.0 (17.8–22.4)</td>
<td>NS</td>
</tr>
<tr>
<td>C</td>
<td>DDVP + MEC1</td>
<td>17.6 (15.3–20.2)</td>
<td>NS</td>
</tr>
<tr>
<td>D</td>
<td>DDVP + MEC10</td>
<td>18.3 (15.6–21.5)</td>
<td>NS</td>
</tr>
<tr>
<td>E</td>
<td>DDVP + MK-801</td>
<td>&gt;35⁵ vs. A, B, C, D*</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>DDVP + ATR + MK-801</td>
<td>&gt;150⁶ vs. A, B, C, D*</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>DDVP + MEC1 + MK-801</td>
<td>&gt;30⁵ vs. A, B, C*</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>DDVP + MEC10 + MK-801</td>
<td>&gt;50⁴ vs. A, B, D*</td>
<td></td>
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</tbody>
</table>

Note. Behavioral seizures were not induced by DDVP or MET in saline- or ATR-pretreated animals (Tables 1 and 2).

Effect of ATR on DDVP- or MET-induced seizures. Pretreatment with ATR at 1.8 mg/kg did not protect mice from DDVP- or MET-induced behavioral seizures in mice. The respective CD₉₀ values for DDVP and MET were 20.0 mg/kg (Table 1) and 5.7 mg/kg (Table 2). Even at a dose of 10 mg/kg ATR did not affect protection against DDVP- or MET-induced seizures (data not shown).

Effect of MEC on DDVP- or MET-induced seizures. Pretreatment with MEC at the dose of 1 mg/kg did not attenuate occurrence of seizures induced by DDVP. The CD₉₀ value for DDVP in mice pretreated with MEC (1 mg/kg) was 17.6 mg/kg (Table 1). However, MEC (1 mg/kg) attenuated MET-induced convulsions, which was expressed by an increase in the MET CD₉₀ value from 5.2 to 6.3 mg/kg (Table 2).

MEC at a dose of 10 mg/kg did not protect mice from DDVP-induced behavioral seizures. The CD₉₀ value for DDVP in animals preinjected with MEC (10 mg/kg) was 18.3 mg/kg versus 19.7 mg/kg for controls (Table 1). On the other hand, pretreatment with 10 mg/kg MEC significantly decreased the vulnerability of mice to seizures induced by MET. Consequently, the MET CD₉₀ value in animals pretreated with MEC (10 mg/kg) was 6.7 mg/kg versus 5.2 mg/kg for controls (Table 2).

Effect of MK-801 on DDVP- or MET-induced seizures. Pretreatment with MK-801 (1 mg/kg) completely prevented the occurrence of behavioral seizures induced by DDVP administered at doses up to 35 mg/kg (Table 1). It should be noted, however, that MK-801 did not afford any protection against DDVP-induced lethality (see below for results).

MK-801 failed to protect mice against the seizure activity induced by MET administration. The CD₉₀ value for MET in MK-801-pretreated mice was 5.6 mg/kg and did not differ from the CD₉₀ value for MET in saline-pretreated animals (5.2 mg/kg, Table 2).

Effect of coadministration of ATR and MK-801 on DDVP- or MET-induced seizures. Concomitant pretreatment with ATR and MK-801 prevented seizures induced by the administration of DDVP at doses up to 150 mg/kg (Table 1). Concurrent pretreatment with ATR and MK-801 had no protective effect on MET-induced convulsions in mice. The CD₉₀ for MET in mice comcomitantly pretreated with ATR and MK-801 was 5.5 mg/kg, which was close to the CD₉₀ of MET in saline- or ATR-pretreated animals (5.2 or 5.7 mg/kg, respectively, Table 2).

Effect of coadministration of MEC and MK-801 on DDVP- or MET-induced seizures. Concomitant pretreatment with MEC (1 mg/kg) and MK-801 blocked the seizure activity induced by the administration of DDVP or MET at doses up to 30 mg/kg (Table 1) and 9 mg/kg (Table 2), respectively. Similarly, concurrent pretreatment with the high dose of MEC (10 mg/kg) and MK-801 completely prevented mice from exhibiting convulsions produced by DDVP or MET at doses up to 50 mg/kg (Table 1) and 20 mg/kg (Table 2), respectively.

2-h Lethality

DDVP- or MET-induced lethality. The ip administration of DDVP or MET resulted in dose-dependent lethality in mice.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Treatment</th>
<th>MET CD₉₀ (95% confidence limit)</th>
<th>p &lt; 0.05</th>
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<tbody>
<tr>
<td>A</td>
<td>MET</td>
<td>5.2 (4.8–5.7)</td>
<td>NA</td>
</tr>
<tr>
<td>B</td>
<td>MET + ATR</td>
<td>5.7 (4.7–6.9)</td>
<td>NS</td>
</tr>
<tr>
<td>C</td>
<td>MET + MEC1</td>
<td>6.3 (5.6–7.1) vs. A, B</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>MET + MEC10</td>
<td>6.7 (6.0–7.4) vs. A, B</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>MET + MK-801</td>
<td>5.6 (5.0–6.4)</td>
<td>NS</td>
</tr>
<tr>
<td>F</td>
<td>MET + ATR + MK-801</td>
<td>5.5 (4.5–6.6)</td>
<td>NS</td>
</tr>
<tr>
<td>G</td>
<td>MET + MEC1 + MK-801</td>
<td>&gt; 9⁴ vs. A, B, C, E*</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>MET + MEC10 + MK-801</td>
<td>&gt;20⁴ vs. A, B, D, E*</td>
<td></td>
</tr>
</tbody>
</table>

Note. Behavioral seizures were induced by methomyl (MET) in mice pretreated with a muscarinic antagonist atropine (ATR, 1.8 mg/kg) or a nicotinic antagonist mecamylamine (MEC1, 1 mg/kg; MEC10, 10 mg/kg) coadministered with a glutamate antagonist dizocilpine (MK-801, 1 mg/kg). CD₉₀ values (expressed in mg/kg) were determined and compared using probit analysis (Litchfield and Wilcoxon, 1949). NA, not applicable; NS, not statistically significant.

⁵, ⁶ Behavioral seizures did not occur even at the highest dose used in this experiment, i.e., 9 or 20 mg/kg, respectively; however at these doses ≥ 90% lethality was observed.

⁴ p < 0.05, as determined using Fisher’s exact test (Finney, 1948).
LD₅₀ values for DDVP and MET, as determined within 2 h, were 22.4 mg/kg (Fig. 1) and 6.4 mg/kg (Fig. 2), respectively.

Effect of ATR on DDVP- or MET-induced lethality. ATR (1.8 mg/kg) markedly reduced the lethality produced by DDVP and MET within the 2-h observation period, which was mirrored by increases in the respective LD₅₀ values of 33.9 mg/kg (Fig. 1) and 28.5 mg/kg (Fig. 2).

Effect of MEC on DDVP- or MET-induced lethality. MEC at 1 mg/kg did not protect mice from DDVP- or MET-induced lethality. Respective LD₅₀ values for DDVP and MET in animals pretreated with MEC (1 mg/kg) were 20.7 mg/kg (Fig. 1) and 6.8 mg/kg (Fig. 2). On the other hand, pretreatment with MEC at 10 mg/kg markedly reduced DDVP- and MET-induced lethality. LD₅₀ values for DDVP and MET in animals pretreated with MEC (10 mg/kg) were 34.6 mg/kg (Fig. 1) and 12.7 mg/kg (Fig. 2), respectively.

Effect of MK-801 on DDVP- or MET-induced lethality. MK-801 (1 mg/kg) afforded protection from neither DDVP- nor MET-induced lethality. Respective LD₅₀ values for DDVP and MET in MK-801-pretreated animals were 22.7 mg/kg (Fig. 1) and 6.3 mg/kg (Fig. 2).

Effect of coadministration of ATR and MK-801 on DDVP- or MET-induced lethality. Concurrent pretreatment with ATR and MK-801 significantly diminished DDVP and MET lethality in mice, in comparison with the animals exclusively pretreated with ATR. LD₅₀ values of DDVP and MET in mice preinjected with MK-801 and ATR were 82.9 mg/kg (Fig. 1) and 70.3 mg/kg (Fig. 2), respectively.

24- and 120-h Lethality

The LD₅₀ values for DDVP and MET in saline- and/or ATR- and/or MEC- and/or MK-801-pretreated mice determined after 24 and 120 h of observation were not statistically different from their respective 2-h LD₅₀ values (data not shown).

DISCUSSION

In our studies, ATR diminished the DDVP- and MET-induced lethality, but not seizures. Low dose of MEC was able
to diminish MET-induced seizures. At the high dose, MEC not only attenuated seizures produced by MET, but also decreased lethality of both AChE inhibitors studied. MK-801 attenuated neither DDVP- nor MET-induced lethality, but prevented DDVP-induced seizures. Coinjection of MK-801 and MEC completely prevented seizures produced by both pesticides. Moreover, MK-801 markedly potentiated the protective effects of ATR and MEC against DDVP and MET lethality.

Experimental and clinical studies have shown that convulsive seizures are among the most frequently occurring symptoms of acute OP or carbamate poisonings (Bardin et al., 1994; Honchar et al., 1983). According to a World Health Organization report, convulsions can be seen within 1 h following systemic administration of DDVP in laboratory rodents (WHO Working Group, 1989). Subcutaneous administration of MET at 5 mg/kg resulted in the occurrence of convulsions in rats (Gupta, 1994). Similarly, in our experiments both anticholinesterases induced dose-dependent seizure activity in mice. An overabundance of ACh in the CNS may manifest as convulsive seizures (Vollmer et al., 1979). Thus, it would seem that the muscarinic antagonist ATR should prevent or diminish convulsions induced by AChE-inhibiting compounds that increase ACh in the CNS. However, ATR at 1.8 and even 10 mg/kg did not protect mice against DDVP- or MET-induced seizures. This observation is paralleled by results from other laboratories and clinical reports evidencing the inability of ATR to protect against seizures associated with OP- or carbamate-induced poisonings (Bardin et al., 1994; McLean et al., 1992). On the other hand, some authors have shown that ATR can prevent or stop seizures induced by anticholinesterases. However, in such cases, ATR was administered at very high doses, e.g., the ED50 of ATR in soman- or sarin-induced convulsions was close to 60 mg/kg (Shih and McDonough, 1999).

It is generally accepted that some symptoms associated with AChE inhibitor-induced intoxication are caused by excessive stimulation of nicotinic receptors. Nonetheless, studies on the role of nicotinic receptors in poisonings induced by OPs or carbamates are relatively few. Some authors have demonstrated anticonvulsant efficacy of the nicotinic antagonist MEC in soman-induced seizures (Shih et al., 1991). In the present study, MEC, both at the low (1 mg/kg) and the high (10 mg/kg) dose, attenuated the convulsions produced by MET, but not those produced by DDVP. This is the first evidence of a beneficial effect of nicotinic antagonists to ameliorate convulsions produced by a carbamate.

Cholinergic mechanisms seem to play an important role in anticholinesterase-related toxicity. However, experimental data suggest that the neurotoxic properties of OPs and carbamates cannot be explained solely by their direct action on AChE. EAA systems have been implicated in the effects of AChE inhibitors in rodents (Shih and McDonough, 1997). Indeed, in the present study, the NMDA antagonist MK-801 (1 mg/kg) prevented the occurrence of seizures produced by the OP pesticide DDVP. This observation is in accordance with other published experimental data. In the earliest study on the involvement of EAA systems in the neurotoxicity of anticholinesterases, MK-801, at doses of 1 or 5 mg/kg, attenuated or completely blocked, respectively, soman-induced seizures in guinea pigs (Braitman and Sparenborg, 1989). There are few reports on the influence of NMDA antagonists upon carbamate-induced convulsions. One of these studies has shown that the NMDA antagonist memantine prevents seizures caused by intoxication with the carbamate insecticide, carbofuran (Gupta, 1994). However, in the present report, MK-801 failed to protect mice from the occurrence of MET-induced convulsions, a finding corresponding to the results of our previous studies (Dekundy et al., 2001). Furthermore, concurrent administration of MK-801 and ATR prevented the occurrence of DDVP- but not MET-induced seizures. Interestingly, EAA antagonist-resistant excitatory postsynaptic currents in rat neocortex were reduced by MEC (Chu et al., 2000). This finding seems to correlate well with our observations, where coadministration of MK-801 and MEC (at either 1 or 10 mg/kg) completely prevented the occurrence of both DDVP- and MET-induced seizures.

Undoubtedly, mortality is the greatest clinical concern related to anticholinesterase-induced poisonings. DDVP exposure is one of the most frequent causes of OP-induced lethal poisonings in humans (Yamashita et al., 1997). Systemic administration of DDVP in rodents usually results in the death of the animals within 1 h (WHO Working Group, 1989). Studies on the effects of MET in laboratory animals are relatively few. However, they provide evidence of high toxicity of this compound in rodents (Gupta, 1994). MET has also been fatally ingested by humans (Lifshitz et al., 1997). Correspondingly, both substances studied—DDVP and MET—produced dose-dependent lethality in mice.

The efficacy of ATR in preventing the lethal consequences of AChE inhibitor-related poisonings has been demonstrated in both clinical and experimental studies (Holstege et al., 1997; Minton and Murray, 1988). Likewise, in the present study, ATR (1.8 mg/kg) protected against the lethality produced by DDVP or MET in mice. It should be noted, however, that some authors have not shown efficacy of even high doses of ATR to protect against the lethal effects of some OPs (Clement, 1994).

Low dose of MEC coadministered with ATR, an AChE reactivator and a carbamate diminished the lethality produced by the OPs soman and diisopropylfluorophosphate (Harris and Stitcher, 1984). Until now, no studies concerning the influence of nicotinic antagonists upon the lethal effects of pesticidal OPs or carbamates have been carried out. In the present study, only the high dose of MEC (10 mg/kg) diminished the lethality of DDVP or MET, whereas the low dose of 1 mg/kg was ineffective.

Pretreatment with MK-801 did not protect mice from DDVP- or MET-induced lethality. This finding is also in agreement with other studies. At 1 mg/kg or higher, MK-801
prevented soman-induced seizures, but not lethality (Sparenborg et al., 1992). On the other hand, MK-801 coadministered with ATR significantly diminished the lethality of OPs and carbamates (Braitman and Sparenborg, 1989; Dekundy et al., 2001). Likewise in the present study, MK-801 markedly enhanced the protective effects of ATR against DDVP- or MET-induced lethality. Coadministration of otherwise ineffective doses of MK-801 (1 mg/kg) and MEC (1 mg/kg) diminished the lethality of DDVP and MET. Injection of a subthreshold dose of MK-801 (1 mg/kg) along with MEC at an effective dose (10 mg/kg) increased the protective activity of the nicotinic antagonist in both DDVP- and MET-induced death. This is the first demonstration of such an interaction.

According to a widely accepted theory on the mechanism of AChE inhibitor-induced neurotoxicity, early increases in ACh levels initiate seizure processes. Secondarily, EAAs are recruited, leading to the occurrence of neuropathological changes and lethality (Shih and McDonough, 1997). However, this theory does not convincingly explain the inability of NMDA antagonists to attenuate anticholinesterase-induced lethality or carbamate-induced seizures. It also does not explain the well-documented inability of ATR to prevent or attenuate the convulsions produced by AChE inhibitors. Reasons for these discrepancies should be sought in the relationships between cholinergic and glutamatergic systems in brain. The first neurochemical alteration caused by AChE inhibitors in many CNS regions is a considerable elevation in ACh level (Shih and McDonough, 1997). ACh, acting through nicotinic receptors, may be involved in the propagation of excitatory postsynaptic potentials (Chu et al., 2000). Additionally, stimulation of presynaptic nicotinic receptors may cause continuous release of ACh and glutamic acid via a positive feedback mechanism (McGehee et al., 1995). It has been demonstrated, that the effects of AChE inhibitors may not only result from overstimulation of nicotinic receptors by ACh, but also from their direct action on nicotinic receptors (Bakry et al., 1988). Moreover, AChE-inhibiting carbamates may modulate ACh-induced ionic current flow, as demonstrated in the study conducted on recombinant α4-containing nicotinic receptors (Zwart et al., 2000). On the other hand, muscarinic receptor activation by the surplus ACh may increase the excitability of NMDA receptors through the enhancement of intracellular calcium signalling (Girod et al., 2000; Lu et al., 1999; Markram and Segal, 1992). These findings may, to some extent, explain the favorable interaction of cholinergic antagonists and MK-801 to ameliorate DDVP- and MET-induced poisonings.

AChE-inhibiting chemicals, whether used as drugs, pesticides, or chemical warfare agents may still endanger human lives. The mechanisms underlying OP and carbamate-induced neurotoxicity seem to involve muscarinic and nicotinic cholinergic systems as well as NMDA receptors. Our results and other published data indicate that combined treatment with cholinergic and NMDA antagonists might be beneficial in the treatment of anticholinesterase-induced poisonings.

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