Anticholinergic and Antiglutamatergic Agents Protect against Soman-Induced Brain Damage and Cognitive Dysfunction

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Soman, a powerful inhibitor of acetylcholinesterase, causes an array of toxic effects in the central nervous system including convulsions, learning and memory impairments, and, ultimately, death. We report on the protection afforded by postexposure antidotal treatments, combined with pyridostigmine (0.1 mg/kg) pretreatment, against these consequences associated with soman poisoning. Scopolamine (0.1 mg/kg) or caramiphen (10 mg/kg) were administered 5 min after soman (1.2 LD50), whereas TAB (i.e., TMB4, atropine, and benactyzine, 7.5, 3, and 1 mg/kg, respectively) was injected in rats concomitant with the development of toxic signs. Atropine (4 mg/kg) was given to the two former groups at the onset of toxic symptoms. Caramiphen and TAB completely abolished electrographic seizure activity while scopolamine treatment exhibited only partial protection. Additionally, no significant alteration in the density of peripheral benzodiazepine receptors was noted following caramiphen or TAB administration, while scopolamine application resulted in a complex outcome: a portion of the animals demonstrated no change in the number of these sites whereas the others exhibited markedly higher densities. Cognitive functions (i.e., learning and memory processes) evaluated using the Morris water maze improved only partially protection. These results show that drugs with a pharmacological profile consisting of anticholinergic and antiglutamatergic properties such as caramiphen and TAB, have a substantial potential as postexposure therapies against intoxication by organophosphates.

Key Words: TAB; caramiphen; scopolamine; soman; Morris water maze; EEG; peripheral benzodiazepine receptors.

Organophosphorous (OP) compounds, such as soman, are powerful inhibitors of acetylcholinesterase (AChE) that cause deleterious central nervous system (CNS) effects (Lemercier et al., 1983; Taylor, 2001). As a consequence of enzyme inhibition, a progression of toxic signs including hypersecretions, tremors, convulsions, respiratory distress, learning and memory impairments, and, ultimately, death ensue (Glenn et al., 1987; McDonough and Shih, 1993). The initial phase of this process is due to massive acetylcholine (ACh) accumulation leading to excessive cholinergic activity (Lallement et al., 1992; Shih, 1982). Thus, the primary targets of protective therapies against OP poisoning were the shielding and reactivation of AChE and antagonizing the effects of ACh on muscarinic receptors. A combined regimen consisting of prophylaxis and therapy is now generally accepted as the ultimate countermeasure against nerve agent poisoning (Dunn and Sidell, 1989; Moore et al., 1995).

The protective efficacy of pretreatment with reversible inhibitors of AChE, for example, pyridostigmine (PYR) (Lennox et al., 1985) in conjunction with anticholinergic agents such as atropine (ATR) and scopolamine (SCO) (Capacio and Shih, 1991; Leadbeater et al., 1985) is well documented. However, while present multidrug treatments for OP intoxication offer robust protection against lethality (Dunn and Sidell, 1989; Moore et al., 1995), seizures and behavioral deficits are not completely prevented (Leadbeater et al., 1985; Shih and McDonough, 1999). These treatments failed to control the convulsions associated with excitotoxicity of glutamate, released following the initial step of excessive cholinergic output (Lallement et al., 1991, 1992). Notably, antagonists of N-methyl-D-aspartate (NMDA) (Braitman and Spenborg, 1989) or AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors (Lallement et al., 1993), two subtypes of central glutamate receptors, afforded additional protection. In this respect, it is important to note that primate poisoned with a high dose of soman (10 or 3 LD50) and then treated with TAB, showed remarkable recovery (Raveh et al., 1997; von Bredow et al., 1991). This antidote is a mixture consisting of the oxime TMB4, atropine, and benactyzine, the latter being a cholinolytic agent with demonstrated antiglutamatergic activity (McDonough and Shih, 1995).

Numerous investigations have shown that exposure to a diverse group of OPs at distinct doses produces neuronal loss in various CNS structures, particularly in the entorhinal and...
the TAB mixture to block the deleterious effects of soman (1.2 LD50) exposure antidotal treatment. The ability of CRM, SCO, and TAB, we examined the TAB mixture, our standard post exposure therapies, will provide a clear increase in the density of peripheral benzodiazepine receptors (PBR, ω3 sites) following CNS injury (Benavides et al., 1987). Moreover, Lallement et al. (1993) and Dematteis et al. (1997) demonstrated that soman-induced seizures are intimately related to a dramatic elevation of PBR-binding capacity. Recent reports by Kuhlmann and Guilarte (1997) and Raveh et al. (2002), suggested that subtle neuronal detriments could be detected by monitoring changes in ω3 sites and cognitive function in nonconvulsing rats. Interestingly, the former report clearly shows that low doses of the glutamatergic agent, domoic acid, which did not cause any morphological alterations, resulted in impaired performance in the Morris water maze tasks concomitant with elevated PBR densities (cf., Weissman and Raveh, 2003).

Spatial learning and memory impairments are two major detrimental consequences of OP intoxication, with ensuing neuronal degeneration in CNS structures (e.g., hippocampus). Indeed, cognitive incapacitation, such as impaired learning and memory resulting from exposure to OPs, has been reported in humans and animals (Blick et al., 1994; Brown and Brix, 1998). The Morris water maze test is known for its sensitivity to hippocampal damage (Brandeis et al., 1989; Hagan et al., 1989) and, as such, is well suited to assess soman-evoked cognitive impairments (Brandeis et al., 1993; Filliat et al., 1999; Raveh et al., 2002).

Recently published results have demonstrated that caramiphen (CRM), a drug with a combined anticholinergic and antiglutamatergic profile, affords better protection when compared to that provided by SCO, a pure anticholinergic agent, when given in a pretreatment paradigm (Raveh et al., 2002). The present study was designed to examine whether SCO and CRM, when used as post exposure therapies, will provide a similar pattern of differential protection. In addition to CRM and SCO, we examined the TAB mixture, our standard post exposure antidotal treatment. The ability of CRM, SCO, and the TAB mixture to block the deleterious effects of soman (1.2 LD50), in conjunction with pyridostigmine pretreatment, is presented herein. To this end, intoxicated animals were examined in the Morris water maze to assess cognitive function, and their brain tissues were tested for proliferating reactive microglia using [3H]Ro5-4864-binding experiments. Additionally, electrocorticogram (ECoG) measurements were undertaken to investigate the effects of these drugs on electrographic seizure activity (EGSA) observed after soman exposure.

**Materials and Methods**

**Materials.** [3H]Ro5-4864 (specific activity 85.4 Ci/mmol) was purchased from NEN DuPont (Boston, MA). Soman (pinacolyl methylphosphonofluoridate) was synthesized by the Department of Organic Chemistry IIBR. Ro5-4864 was the generous gift of Dr. A. H. Newman (NIDA, Baltimore, MD) and was dissolved in absolute ethanol. Caramiphen edisylate was purchased from Secifarma, Italy. Pyridostigmine hydrobromide, TMB4, scopolamine hydrochloride, atropine sulfate, benactyzine, and all other chemicals were purchased from Sigma-Aldrich (Ness Ziona, Israel).

**Subjects.** Male Sprague-Dawley rats (2.5–3 months old, 250–330 g) were obtained from Charles River (U.K.) and were housed, five per cage, in a controlled environment (12 h light/dark cycle, 22 ± 1°C) with water and food ad libitum. Rats were maintained in accordance with the principles enunciated in the Guide for the Care and Use of Laboratory Animals, NAS 1996 publication, seventh ed., Washington, DC.

**Study design.** Rats were treated with PYR (0.1 mg/kg, im) and randomly divided into the following experimental groups:

- a. Control: Atropine (4 mg/kg, im) was administered 20 min after PYR (n = 16).
- b. Soman/atropine: Injected with soman (108 mg/kg, im, 1.2 LD50) 20 min after PYR. Atropine (4 mg/kg, im) was administered concomitant with the appearance of intoxication signs such as fasciculation and tremors (2–4 min) after PYR (n = 29).
- c. Soman/TAB: TAB (7.5, 3 and 1 mg/kg for TMB4, atropine, and benactyzine, respectively) was administered, im, at onset of symptoms after soman exposure (n = 29).
- d. Soman/atropine/caramiphen: Rats treated as in (b) above, and CRM (10 mg/kg) injected 5 min after soman exposure (n = 37).
- e. Soman/atropine/scopolamine: Rats treated as in (b) above, and SCO (0.1 mg/kg, im) injected 5 min after soman exposure (n = 66).

The paradigm for TAB administration was adopted from von Bredow et al. (1991) and Raveh et al. (1997), whereas SCO and CRM were examined following several previously reported experimental protocols (e.g., McDonough et al., 2000). Animals were under constant observation for the first 8 h. Rats from all of these groups (n = 3–5) were decapitated at 48 h or 7 days following the last treatment for [3H]Ro5-4864-binding experiments. Behavioral testing was performed between 9:00 a.m. and 1:00 p.m. at 1 week following treatments, and was continued for 5 days. At 1 week following the starting date of the behavioral experiments, rats were decapitated and their brains prepared for [3H]Ro5-4864 binding.

The doses of CRM and SCO used herein had been shown to provide marked protection against soman poisoning (1 LD50) when given prophylactically (Raveh et al., 1999, 2002).

**ECoG recordings.** Animals were implanted 1 week before soman exposure, with chronic cortical stainless-steel electrodes with a miniature plug (Plastic One, UK) and anchoring stainless-steel screws, while under Equithesin anesthesia (chloral hydrate, MgSO4, propylene glycol, nembutal, and ethanol). The electrode holes were drilled approximately equidistant between bregma and lambda and bilaterally ±4.0 mm from the midline. The screws and electrodes were then anchored to the skull and insulated with dental acrylic.

Electrocorticographic recordings were made using hardware and software of Biopac Systems, Inc. (Santa Barbara, CA). During ECoG recordings, animals were housed in individual net-insulated plastic recording chambers that allowed free movement, with the recording leads attached to their electrodes via a nonfriction swivel connector on top of the rats’ heads.

**Binding Experiments.**

**Tissue preparation for [3H]Ro5-4864 binding.** The membrane suspension used for these experiments was prepared as described by Weissman et al.
(1984). Briefly, rats were killed by decapitation and their brains rapidly removed and placed in ice-cold saline. Brains without cerebellum and brain stem (i.e., forebrain/midbrain) were weighed and homogenized in 30 volumes of 50 mM Tris–HCl buffer (pH 7.4) using Polytron (Ultra Turrax, 15 s, setting 5–6). The homogenate was centrifuged for 20 min at 4°C and 20000 × g. The supernatant was discarded and the pellets resuspended in 40 volumes of Tris–HCl buffer.

**Binding of [3H]Ro5-4864 to rat brain membranes.** Binding of [3H]Ro5-4864 to rat brain membranes was performed as previously described (Weissman et al., 1984) with minor modifications. Briefly, reaction mixtures (in 12 × 75 test tubes), at a final volume of 0.5 ml, contained 50 µl Tris–HCl buffer (50 mM, pH 7.4), 25–µl membrane suspension (300–500 mg protein), 50 µl [3H]Ro5-4864 solution (six ligand concentrations, 0.25 to 6.0 nM), and 50 µl Ro5 4864 solution (10 µM final concentration, for nonspecific binding) or buffer. Nonspecific binding amounted to 5–15% of total ligand bound. Tubes were incubated for 1 h at 0–4°C and the reaction terminated by rapid filtration over GF/B filters. Radioactivity was assessed as described above. Protein content of the binding assay tubes was determined by the Bradford method, using BSA as standard (Bradford, 1976).

Thirty-eight of the rats taking part in the behavioral experiment (38/65) were randomly selected and sacrificed two weeks after soman exposure, and their brains excised and prepared for [3H]Ro5-4864 binding. Forebrain/midbrain membrane suspensions were incubated with a single concentration of [3H]Ro5-4864 (1 nM), whereas other experimental details were as described above.

**Behavioral Experiments**

**Apparatus.** Rats were tested in a circular water maze (diameter: 1.4 m; height: 50 cm) that was painted white and filled to a height of 25 cm with water (26 ± 1°C) in which powdered milk was dissolved. An invisible white metal platform (12 × 12 cm) covered by wire mesh was present inside the pool; its top surface was 20 mm below the surface of the water. Thus, the platform was invisible to a viewer inside the pool. The pool surface was divided into four quadrants of equal area, NE, NW, SE, and SW. The platform was placed midway between the center and rim of the pool in any of the four quadrants. The maze was brightly lit and surrounded by well-lit, salient objects, which were held constant throughout training.

Performance in the maze was monitored by a tracking system consisting of an overhead video camera linked to a TV monitor and an image analyzer (CIS-2) coupled to a microcomputer (system designed and produced by Galil Laboratories, Ltd., Migdal Ha-Emek, Israel). The platform was located in quadrant No. 1.

**Procedures**

**Training.** Each rat was trained for 4 days, 4 trials (1 block/day), in which the platform position remained constant and was located in the center of the northwest quadrant of the pool. Within each block of four trials, each rat started at each of the starting locations (NE, SE, NW, and SW), but the sequence of locations was randomly selected. A trial consisted of placing a rat by hand into the water facing the wall of the pool at one of four starting locations around the pool’s perimeter. Prior to training, the rat was placed on the platform for 60 s. If subsequently on a particular trial, a rat found the platform, it was allowed to remain on it for 60 s. A trial was terminated after 120 s if a rat failed to find the platform, and the rat was placed on the platform for an additional 60 s before starting the next trial. Escape latency (the time to find the platform), path length (the distance traveled by the rat), and speed (the swimming rate of the rat) were recorded on each trial by the monitoring system.

**Probe trial.** Three min following the last training trial (trial 16), the platform was entirely removed from the pool. In this trial (trial 17), each rat was placed in the water (starting location, W) for a limited period (30 s) and its spatial bias was measured by recording the relative distribution of time spent and path length over the four quadrants of the pool.

**Reversal test.** During trials 18 through 21 (fifth day), the platform position was changed to the southeast quadrant, opposite to the training quadrant. Thus, during reversal learning, the platform location was moved relative to the configuration of objects within the room, but the pool occupied the same place within the room throughout the entire experiment. Rats were evaluated as described above.

**Data analysis.** Binding parameters were assessed by computer-assisted nonlinear, least-square regression analysis, using the Prism2 program (GraphPad Software, Inc.) to fit the equation:

$$B = \frac{B_{\text{max}} + T + K_d}{2} - \left(\frac{\left(B_{\text{max}} + T + K_d\right)}{2} - B_{\text{max}}T\right)^{1/2}$$

where B is the amount of bound [3H]Ro5-4864 [in disintegration per minute (dpm)], Bmax is the maximal binding density for [3H]Ro5-4864, T is the total concentration of [3H]Ro5-4864 (in dpm), and Kd is the dissociation constant of Ro5-4864. This equation derives from the basic equilibrium formula

$$(L)[B]_{\text{max}} = K_c$$

under the specialized condition where free ligand concentration (L) is significantly less than T, due to formation of complexes, B.

In the behavioral experiments escape latency, path length, and swimming speed of the four trials on each of the training days were grouped into blocks (one block for each day). Training and probe tests were analyzed by MANOVA, while the scores of reversal test were analyzed by ANOVA, utilizing SPSS software (version 11). Specific comparisons were performed using the simple main effects, contrasts analysis, or the Bonferroni test for multiple comparisons. For clarity of figures, only the lowest significance levels are shown.

**RESULTS**

**Clinical Observations**

Manifestations of symptoms (e.g., tremors, salivation, tonic-clonic convulsions, and respiratory distress) in soman-treated rats appeared within 2 to 4 min after injection. Administration of ATR at the onset of toxic signs postponed the time of death. The mortality rate (24 h score) was 13/38 in the soman/ATR group. These rats experienced convulsions that lasted for several h and in some cases, episodes of convulsions were noticed throughout the week of observation. In contrast, all the rats treated with caramiphen and ATR or TAB demonstrated an immediate termination of convulsive activity, as reported by observers and according to ECoG recording (Table 1). The mortality rates of these animals were 9/37 and 7/29, respectively; most of the rats died before treatment was applied. Antidotal therapy with ATR and SCO yielded a more complex pattern, namely, convulsions were abolished only 10 min after SCO injection in one half of the rats. In the other set of rats, convulsions continued for longer periods (>1 h). No adverse behavior was noted in animals from this group during the next days of observation and the survival rate was 22/66. It should be noted that a mortality rate of 20–30% of the population was observed during the course of initial ECoG recording sessions in the three treatment regimens.

The weights of the animals participating in the behavioral studies (n = 65) were monitored during the week following intoxication and post treatment. Weight loss reached its maximal level (19%, p < 0.001) 72 h following intoxication in soman/ATR animals. One week following poisoning, these rats still showed a 10% loss of weight (p < 0.001). All treated rats showed maximum weight loss at 24 h following exposure (14,
TABLE 1
Percentage of Animals Exhibiting EGSA at Various Times after Exposure to Soman

<table>
<thead>
<tr>
<th>Time postexposure</th>
<th>Atropine</th>
<th>Atropine + scopolamine</th>
<th>Atropine + caramiphen</th>
<th>TAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 (min)</td>
<td>83</td>
<td>66</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30 (min)</td>
<td>83</td>
<td>33</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>60 (min)</td>
<td>83</td>
<td>33</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3 (h)</td>
<td>83</td>
<td>33</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24 (h)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Note. Rats were exposed to 1.2 LD_{50} of soman and treated with various antidotes, as described in Materials and Methods. ECoG recording was performed in groups of six rats.

8, and 6% in SCO, CRM, and TAB, respectively; \( p < 0.05–0.001 \); a significant weight gain was observed 1 week later.

ECoG recordings. Rats exposed to 1.2 LD_{50} of soman developed electrographic seizure activity (EGSA) within 2–5 min after exposure. Similar observations of soman-induced seizures were described previously in some animal models (e.g., Koplovitz and Skvorak, 1998). As shown in Table 1, most animals (83%) of the soman/ATR group (i.e., group b), concomitant with the development of toxic signs, developed EGSA that lasted for hours (Table 1). A typical electrographic record of an atropine-treated rat is depicted in Figure 1A. In animals treated with a combination of atropine and scopolamine (i.e., group e), EGSA was common (66%) in proximity to soman exposure (10 min), and less common later (30–180 min postexposure) (Table 1). Two examples of ECoG records of scopolamine-administered rats are presented in Figures 1C and 1D, demonstrating that this group experienced a degree of seizures greater than TAB or CRM, but less than the soman/ATR group. Treatment with TAB or atropine and caramiphen (i.e., groups c and d); resulted in abrupt termination of EGSA following soman exposure in most cases (Table 1). A typical cessation of EGSA by CRM and ATR is shown in Figure 1B. Figure 1E portrays a representative recording of a TAB-injected rat that is reminiscent of Figure 1B (CRM and ATR). In addition to the observed instantaneous stopping of EGSA, a long-lasting reduction in electrographic activity is evident. Twenty-four h following intoxication, no EGSA could be detected in any experimental group (Table 1). Nevertheless, episodes of abnormal electrographic activity were present at this time point in soman-exposed animals treated only with ATR and in part of the rats receiving ATR and SCO (data not shown).

Behavioral Experiments

General considerations. ECoG recordings and \([^{1}H]Ro5-4864-binding data (see below) suggested the existence of two classes in scopolamine-protected animals. Similar division was also evident from behavioral experiments, thus producing SCO-I and SCO-II subgroups. The criteria for this division was the mean score (i.e., of escape latency as well as path length) of the control group, namely, animals scoring equally or lower than these measures were designated SCO-I subgroup, while rats with scores higher than the control mean were defined as SCO-II animals. The numbers of rats in the different experimental groups were as follows: control, 11; soman, 12; SCO-I, 9; SCO-II, 12; CRM, 12; and TAB, 9.

Escape latency and path length. The interaction between treatment and days was statistically significant, \(F(15/177) = 2.01, p < 0.017\) and \(F(15/177) = 3.03, p < 0.0001\) for escape latency and path length, respectively. The escape latency of the soman/ATR group was significantly longer when compared to all other groups (\(p < 0.01–p < 0.001\)) throughout the training period (Fig. 2). The escape latency of SCO-II rats was significantly longer than that of control rats (\(p < 0.05–p < 0.001\)), while that of TAB-treated rats was longer than that of control rats during the first two days only (\(p < 0.05–p < 0.001\)). Rats receiving CRM showed a similar deficit only on the first day, whereas the performance of the SCO-I group did not differ from that of control animals. The path length of soman/ATR-injected rats was significantly longer throughout the training period relative to control or drug-protected rats (\(p < 0.01–p < 0.001\)) (Fig. 3), excluding the first day when this group performed as did the SCO-II subjects. The path length of SCO-II rats was as described for escape latency. The path length of TAB-treated rats was longer than that of control rats on the second day of training (\(p < 0.001\)). Caramiphen and SCO-I rats performed as did control rats (Fig. 3).

Swimming speed. The interaction between treatment and days for this parameter was found significant, \(F(15/177) = 2.21, p < 0.008\). The swimming speed of the soman/ATR group was considerably slower than that of the control or treated groups (\(p < 0.01–0.001\)) during the entire training period (Fig. 4). On the first day of training, CRM and both SCO groups were slower than control rats (\(p < 0.01–0.001\)). On the second day, however, only caramiphen rats were still deficient in this measure. Overall, it was observed that the swimming speed of control rats remained stable throughout the training, that of the soman/ATR group improved on the second day and did not change thereafter, and speed of the protected groups exhibited improvement during most of the training period.

FIG. 1. ECoG tracing of rats exposed to 1.2 LD_{50} of soman and treated with various antidotal therapies as described in Materials and Methods. Recordings were carried out as described in Materials and Methods. Each panel depicts a representative record: (A) animal treated with ATR, (B) ATR and CRM combination, (C) ATR and SCO regimen (SCO-I), (D) ATR and SCO regimen (SCO-II), and (E) an animal that received TAB.
Probe trial. The interaction between treatment and quadrants of the pool was found statistically significant, F(15/177) = 3.01, p < 0.0001 and F(15/177) = 3.75, p < 0.0001 for time spent and path length, respectively. Soman/ATR-exposed animals did not show any spatial bias during the probe trial; the time spent and the distance swum was equal in each of the four quadrants of the pool (Figs. 5 and 6). In marked contrast, control as well as protected rats spent more time and swam longer distances in the training quadrant than in the other three quadrants (p < 0.05–0.001). Interestingly, SCO-II animals could just differentiate between the training quadrant and two other quadrants in the time-spent measure. Thus, unprotected rats did not demonstrate any tendency to search for the platform during the probe trial, while protected rats displayed a spatial bias similar to control. Yet, SCO-II rats disclosed a partial spatial bias only.

Reversal test. The treatment main effect was found to be statistically significant, F(5/59) = 11.42, p < 0.0001, F(5/59) = 5.97, p < 0.0001 and F(5/59) = 3.37, p < 0.01, for escape latency, path length, and swimming speed, respectively. Rats exposed to soman and ATR manifested impaired performance relative to all other groups, as indicated by both escape latency (p < 0.0001) and path length (p < 0.046–0.001) measures (Figs. 2 and 3). While the swimming speed of the CRM group was significantly higher than that of soman/ATR rats (p < 0.039, Fig. 3), no additional differences were recorded.

Binding Experiments

Pyridostigmine and atropine do not produce any changes in the number or the affinity of sites in the adult rat brain (results not shown). Forty-eight h and 7 days following soman
intoxication (1.2 LD50; 4 mg/kg ATR concomitant with the appearance of intoxication signs-soman/ATR group), the maximal binding capacity of [3H]Ro5-4864 binding in rat forebrain/midbrain homogenates was increased by 182 and 195%, respectively (p < 0.001), compared to control animals (Table 2). Similar changes were observed in SCO-injected animals that exhibited convulsive activity (SCO-II, 207 and 213%, respectively). Caramiphen and TAB regimens completely abolished the elevation in the density of PBR (Table 2). Statistical analysis demonstrated a significant effect of the treatments and showed that the Bmax values of the TAB, CRM, and SCO-I groups were different from those of the ATR and SCO-II rats (p < 0.001). It should be noted that the PBR densities of the former three groups were not statistically different from control levels. In addition, there were no significant differences between the Bmax values of [3H]Ro5-4864 of the various groups at 48 h compared to those measured seven days after soman exposure. Although the Kd values of [3H]Ro5-4864 binding fluctuated between 1.10 and 2.95 nM (Table 2), no statistical analysis on this parameter was carried out, since changes in its value have no physiological relevance.

**Correlation**

A Pearson correlation analysis was performed between learning measures, namely, the mean score during the four training days, versus [3H]Ro5-4864 binding to glial cells. The correlation coefficients were computed on 38 of the animals (n = 65; 58%), randomly selected from the five experimental groups on which both behavioral and binding data were recorded. Positive correlations were found between the extent of neuronal damage as expressed by [3H]Ro5-4864 binding and escape latency [r (36) = 0.72, p < 0.001] as well as path length [r (36) = 0.73, p < 0.001].

**DISCUSSION**

The present study examined the effectiveness of three post-exposure therapies against soman (1.2 LD50) poisoning following PYR pretreatment. Atropine sulfate, administered at the development of intoxication signs, was a common component of these treatments, whereas the oxime TMB4 was present in the TAB mixture only. While the three therapies included an anticholinergic drug (i.e., SCO, CRM, or benactyzine), TAB and CRM possess antiglutamatergic properties. It should be noted that the doses of scopolamine and caramiphen used in this investigation provided excellent shields against soman-induced adverse effects and mortality when administered prophylactically (Raveh et al., 1999, 2002). Results reported herein indicate that while all these antidotal mixtures afford considerable protection, CRM and TAB emerge as the preferred approach compared to that offered by SCO. Although no dramatic differences could be observed in mortality rates, the inferior action of SCO was evident from the ECoG recordings, PBR densities, and behavioral measures.

As expected, the applied dose of soman caused EGSA and violent convulsive activity in most animals (Table 1, Fig. 1). Conversely, none of CRM- or TAB-administered rats exhibited any abnormal electrographic activity following antidotal treatment. Scopolamine-injected animals displayed a mixed pattern, namely, two-thirds of the rats demonstrated EGSA 10 min after soman intoxication while no EGSA was recorded in the

**TABLE 2**

Parameters of the Binding of [3H]Ro5 4864 to Rat Forebrain/Midbrain Homogenates 48 h or 7 Days following Soman Intoxication

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Kd for 48 h (nM)</th>
<th>Bmax for 48 h (fmol/mg prot.)</th>
<th>Kd for 7 days (nM)</th>
<th>Bmax for 7 days (fmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.10 ± .09 (5)</td>
<td>273.2 ± 24.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>ATR</td>
<td>2.01 ± .14 (5)</td>
<td>771.0 ± 32.1*</td>
<td>2.95 ± .07 (4)</td>
<td>805.2 ± 62.9*</td>
</tr>
<tr>
<td>TAB</td>
<td>1.33 ± .11 (4)</td>
<td>261.0 ± 20.0**</td>
<td>1.66 ± .03 (3)</td>
<td>288.9 ± 10.75</td>
</tr>
<tr>
<td>ATR + CRM</td>
<td>1.33 ± .18 (5)</td>
<td>300.5 ± 36.8**</td>
<td>1.33 ± .18 (5)</td>
<td>289.0 ± 11.8</td>
</tr>
<tr>
<td>ATR + SCO I</td>
<td>1.21 ± .06 (5)</td>
<td>312.1 ± 53.3**</td>
<td>1.11 ± .22 (4)</td>
<td>364.4 ± 30.4</td>
</tr>
<tr>
<td>ATR + SCO II</td>
<td>2.30 ± .15 (5)</td>
<td>840.0 ± 39.9*</td>
<td>1.28 ± .15 (3)</td>
<td>855.1 ± 132.7*</td>
</tr>
</tbody>
</table>

*Note. Parameters of [3H]Ro5 4864 binding were determined as described in Materials and Methods. Experiments were performed in duplicate and the results are expressed as means ± SEM with the number of animals in each group in parentheses.

*Significantly different from control; p < 0.001.

**Significantly different from ATR and ATR + SCO II; p < 0.001.
rest. Notably, the same dose of SCO, given as a pretreatment, yielded complete prevention of seizure activity caused by soman (1 LD₅₀) (Raveh et al., 1999).

Additional support for the distinct profiles of the drugs used in this research is derived from [³H]Ro5-4864 binding studies. Our results show that soman/ATR rats experience a marked (~3-fold) elevation in the number of binding sites for this ligand that is maximal 48 h after intoxication (Table 2). Carumphen and TAB abolished the robust increase in PBR density while SCO effects were complex; i.e., animals treated with this antimuscarinic agent, could be divided into two sets. Unlike CRM or TAB, scopolamine prevented the increase in this sensitive and reliable marker for neuronal injury and glial proliferation (Weissman and Raveh, 2003) in one half of the subjects examined.

In accord with a recent report (Raveh et al., 2002), rats exposed to 1.2 LD₅₀ of soman sustained severe deficits in three cognitive processes (i.e., learning, memory, and reversal learning), as measured in the Morris water maze task. Similar behavioral impairments in learning and spatial memory were correlated to brain pathology in general and degeneration in the hippocampal structures in particular (Carpentier et al., 1991; Filliat et al., 1999).

The three antidotal regimens employed considerably improved learning performance in soman-injected animals. However, there was a clear hierarchy in terms of the ability of these therapies to affect learning, namely, SCO II << TAB < CRM < SCO I. In the memory test, which reflects the spatial strategy of the rats (McNamara and Skelton, 1993), all the animals showed complete spatial tendency similar to control rats, except for the SCO-II group. Nonspecific motor coordination effects could explain neither the behavioral deficit of soman/ATR rats nor the improving effects of SCO, CRM, or TAB, because changes in swimming speed did not consistently correlate with cognitive effects.

The degree of protection against cognitive dysfunction afforded by CRM and TAB treatments is reminiscent of that to human butyrylcholinesterase, a therapeutic regimen shown to provide excellent protection against soman poisoning (Brandeis et al., 1993). However, data presented herein show that SCO does not reach the potency of either TAB or CRM. In contrast to CRM or TAB, the protective potential of SCO (at the dose examined) is heterogeneous. The division of the SCO group into SCO-I and SCO-II subsets, according to their learning profile, place the SCO-I group at a similar efficacy stage as CRM and TAB. Nevertheless, SCO-II rats exhibited marked deficits in every parameter examined, placing them at the bottom of the antidotal potency scale. The different responses to SCO application may be due to the existence of distinct patterns of the development of intoxication signs. Thus, SCO may provide better defense against seizures if given at the first phase following soman exposure (i.e., the cholinergic phase), prior to the engagement of the excitotoxic amino-acid system that leads to a massive activation of glutamate receptors.

Examining the correlations between learning parameters (i.e., escape latency and path length) and brain damage, as estimated by the binding capacity of [³H]Ro5-4864 to forebrain/midbrain membranes, reveals relatively high values (r = 0.72 and r = 0.73). These values imply that 52 and 53%, respectively, of the variance in learning parameters might be accounted for by the changes measured in PBR density. These levels of correlation are slightly higher than those observed for prophylactic use of SCO and CRM (Raveh et al., 2002) and considerably greater than those reported by Filliat et al. (1999). Thus, it can be concluded that the number of α3 sites is a valuable marker for both brain damage and cognitive function.

Recent reports (Raveh et al., 1999, 2002) indicated the involvement of NMDA and AMPA, two subtypes of the glutamate receptor family, in processes associated with soman exposure. Similarly, previous accounts by Lallement et al. (1991, 1992) demonstrated the presence of an intimate relationship between the cholinergic system activated by ACh, accumulated due to AChE inhibition and alterations of various glutamate receptors. In fact, McDonough and Shih (1997) stated that a glutamatergic stage follows the initial cholinergic over-secretion/stimulation stage. Thus, it was hypothesized that compounds with antiglutamatergic characteristics could be beneficial in OP poisoning. Notably, some NMDA and AMPA antagonists were shown to afford considerable protection against soman poisoning (Lallement et al., 1993, 1994; McDonough and Shih, 1997). Since benactyzine and CRM had been shown to possess such properties (McDonough and Shih, 1995; Raveh et al., 1999) combined with potent anticholinergic characteristics, their efficacy in this paradigm is logical and evident. Nonetheless, it is possible that applying higher doses of SCO or ATR could result in a better protection profile.

In conclusion, we demonstrated that antidotal therapies based on centrally acting drugs, exhibiting both anticholinergic and antiglutamatergic properties, offer an adequate response to the deleterious consequences of OP poisoning. An improved regimen may emerge from the study of novel candidates possessing these characteristics. Additionally, effort should be directed towards the investigation of temporal relationship between treatment and severity of intoxication signs.

REFERENCES


