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Behavioral Effects of Toxic Doses of Soman, an Organophosphate Cholinesterase Inhibitor, in the Rat: Protection Afforded by Clonidine

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BUCCAFUSCO, J. J., J. H. GRAHAM AND R. S. ARONSTAM. *Behavioral effects of toxic doses of soman, an organophosphate cholinesterase inhibitor, in the rat: Protection afforded by clonidine.* PHARMACOL BIOCHEM BEHAV **29**(2) 309–313, 1988.—Atropine, a postsynaptic muscarinic antagonist, and clonidine, a presynaptic inhibitor of acetylcholine release, protect mice from the lethal effects of soman, a potent and irreversible cholinesterase inhibitor. The purpose of this study was to determine the effects of atropine (6 mg/kg) and clonidine (0.2 mg/kg) on soman-induced lethality and behavioral changes in the rat. Soman produced a dose-dependent increase in lethality over a narrow concentration range (50–200 μg/kg, SC). Soman produced time- and dose-dependent increases in tremor, salivation, hind limb extension, convulsions and chewing behaviors, as well as decreases in three normal stereotyped behaviors, sniffing, locomotion and rearing. Atropine and clonidine were equally effective at limiting soman-induced lethality and behavioral changes. The protective effects of clonidine and atropine were synergistic, even though clonidine antagonizes some of the stereotyped behaviors elicited by atropine. Simultaneous pretreatment with clonidine and atropine completely eliminated the lethality and behavioral changes produced by injection of 200 μg/kg soman.

Organophosphate cholinesterase inhibitor Soman Behavioral toxicity Clonidine Atropine Acetylcholinesterase

CLONIDINE is a centrally-active alpha$_2$-adrenergic agonist which is employed clinically as an anti-hypertensive agent. The most common side effects associated with clonidine therapy are symptoms which normally accompany anti-muscarinic medication, such as dry mouth, sedation, and gastrointestinal and visual disturbances. The ability of clonidine and related drugs to evoke anticholinergic side effects may be related to their inhibition of acetylcholine release from preganglionic autonomic and postganglionic parasympathetic nerve terminals [9]. We have recently shown that clonidine also inhibits acetylcholine synthesis and release in certain brain regions [2–4]. This presynaptic action may prove useful in blocking the symptoms of cholinergic overstimulation associated with cholinesterase inhibition.

In support of this possibility is a study in which we demonstrated that clonidine protects mice from physostigmine, a reversible cholinesterase inhibitor [3]. Clonidine's protective actions were associated with a mitigation of the increase in brain acetylcholine induced by physostigmine [3]. That the protection involved primarily central cholinergic systems was indicated by clonidine's failure to protect against the toxic effects of the peripherally acting cholinesterase inhibitor, neostigmine. More recent studies employing the organophosphate cholinesterase inhibitor, soman, substantiated the physostigmine studies [1,5]. Moreover, the combined use of atropine and clonidine in the pretreatment regimen was found to enhance survival following soman administration. In most cases, the shift in the LD_{50} was greater than a simple additive effect [5]. It was also noted that clonidine-pretreated mice which survived LD_{50} doses of soman had fewer behavioral side effects than mice which did not receive clonidine. The purpose of this study was to determine the relative efficiency of clonidine and atropine at inhibiting (1) soman-induced behaviors and (2) soman-induced suppression of stereotyped behaviors in rats.

METHOD

Male, outbred Wistar rats were obtained from Harlan Sprague-Dawley, Inc. and housed in our animal facilities for

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TABLE 1

EFFECT OF ATROPINE AND CLONIDINE ON SOMAN-INDUCED LETHALITY

Pretreatment (dose, mg/kg)	Percent Lethality	N
Saline	100	11
Atropine (6)	40*	10
Clonidine (0.2)	43*	7
Clonidine (0.5)	0*	6
Atropine (6) + Clonidine (0.2)	0*†‡	6

Rats were pretreated with protective agent 30 min prior soman (0.16 mg/kg). Animals were observed for 24 hours.

*=Significantly different ($p<0.05$) compared to saline controls.

†=Different at the $p<0.1$ level from atropine value.

‡=Different at the $p<0.1$ level from the clonidine (0.2) value.

at least one week prior to the experiment. The animals were maintained on a 12 hr:12 hr light:dark cycle and had free access to tap water and standard chow (Wayne Rodent Blox).

At 9–12 weeks of age, rats were habituated in individual observation cages (44×24×20 cm) located in a quiet room for 2.5 hr just prior to the experiment. Groups of 3 rats were observed for 60 sec every 3 min during a 30 min trial. The presence of the following behaviors/symptoms were recorded at each observation period: tremor, excessive salivation, hind limb extension, convulsions/jerks, chewing, sniffing, locomotor activity and rearing. Other behaviors noted during the observation period included flat body posture, reciprocal forepaw movement, whole body shiver and gnawing/licking; however, these behaviors did not exhibit a dose- or time-effect relationship to soman, or were observed too infrequently to be included in the experimental analysis. After one drug-free observation period (time 0), rats received one of the following treatment regimens: (1) saline-soman, (2) atropine-soman, (3) clonidine-soman, (4) clonidine-atropine-soman. Atropine, clonidine and saline were administered 30 min prior to soman, and clonidine was administered 5 min prior to atropine in regimen 4. All drugs were dissolved in saline and administered subcutaneously (SC).

Data was compared using χ^2 analysis for grouped data as well as for *post hoc* analysis. In all experiments, results between groups were considered significantly different at the $p<0.05$ level.

Atropine sulfate was purchased from Sigma Chemical Co. (St. Louis, MO), clonidine was obtained from Research Biologicals, Inc. (Wayland, MA), and soman (pinacolyl methylphosphonofluoridate) was supplied by the U.S. Army Medical Research and Development Command.

RESULTS

Soman-Induced Lethality in the Rat

Our previous studies [5] indicated that clonidine reduces the lethal action of soman in mice, and that synergistic protective effects were obtained with atropine. The present results in rats confirmed these findings (Table 1); clonidine produced a dose-related reduction in soman-induced lethality and enhanced the protective actions of atropine. The combination of atropine and the low dose of clonidine eliminated lethality, an effect which was different at the $p<0.1$ level from either atropine or clonidine alone and different from saline controls ($p<0.05$).

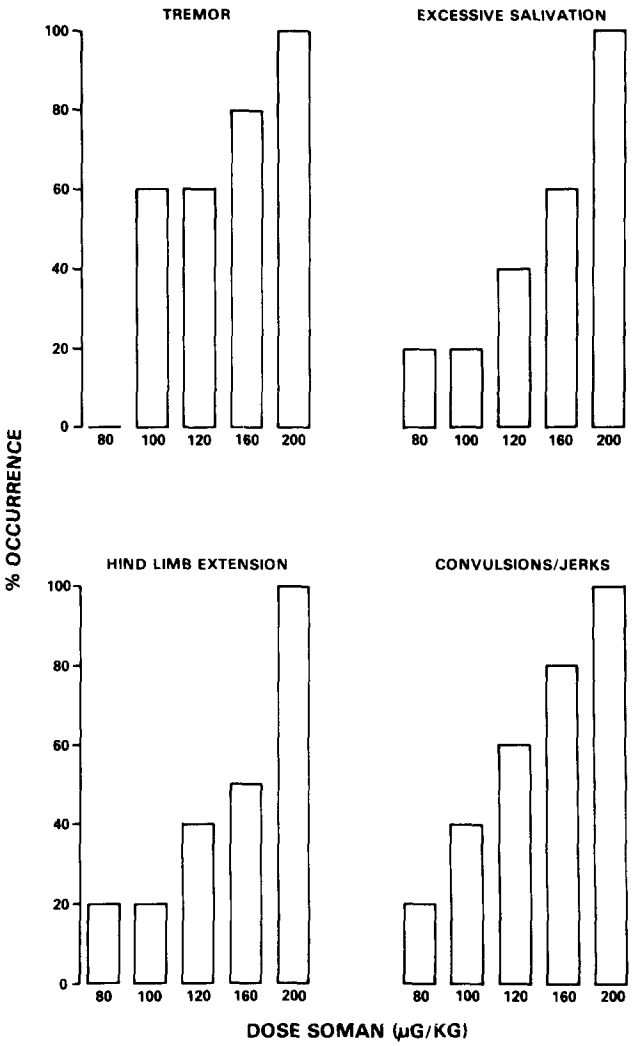

FIG. 1. Effect of increasing doses of soman on the appearance of toxic symptoms. The frequency of the symptoms was maximal for all doses within 12 min after injection and was maintained throughout the 30 min trial. These symptoms were never observed in control animals. The data for the 12 min observation period are presented. N=6–8 per group. There was a significant difference between doses over time ($p<0.05$).

Acute Toxic Symptoms and Behavioral Signs Produced by Soman

Soman produced a significant dose-dependent increase in the occurrence of tremor, salivation, hind limb extension, convulsions/jerks and chewing, and a dose-dependent decrease in normal behaviors such as sniffing, locomotor activity and rearing (Figs. 1 and 2). In each case, the appearance of a sign or symptoms was observed in all animals or, in the case of suppression of ongoing behaviors, the behavior was not observed when the dose of soman was increased to 200 µg/kg. Dose-response curves were extremely steep, occupying a range of only 100–200 µg/kg. The curves may be

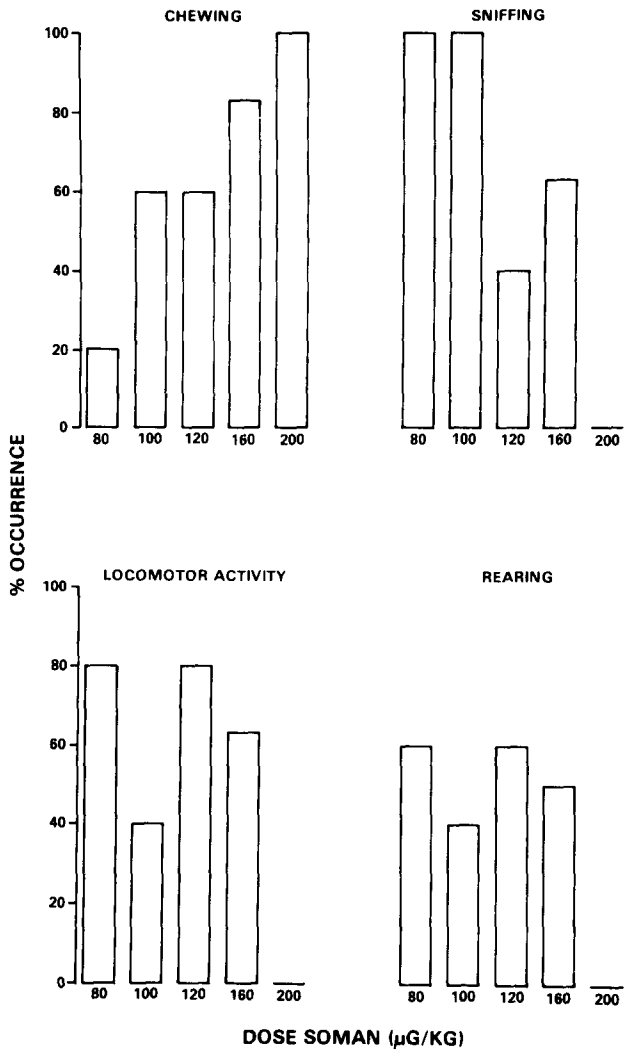

FIG. 2. Effect of increasing doses of soman on stereotyped behaviors. The effect of soman on each behavior was maximal for all doses within 6 min after injection and was maintained throughout the 30 min trial. The data for the 6 min observation period are presented. Chewing was not observed in control animals; the other behaviors were noted in 80–100% of controls. There was a significant difference between doses over time ($p<0.05$).

even steeper for suppression of sniffing, locomotor activity and rearing.

The time-dependence of soman-induced behavioral changes and toxic symptoms is summarized in Figs. 3 and 4. The appearance of tremor, excessive salivation, hind limb extension and convulsion were maximal by 12 min after injection of 200 μg/kg soman, while the incidence of chewing was maximal within 6 min of soman injection. Soman depression of sniffing, locomotor and rearing activities was maximal by 6 min after injection of soman (Fig. 4). In each case, once the maximal effect was attained, there was no decrease in soman-induced behavioral changes over the remainder of the 30 min observation period.

Effect of Atropine and Clonidine on the Behavioral Toxicity to Soman

Pretreatment with clonidine produced a dose-dependent

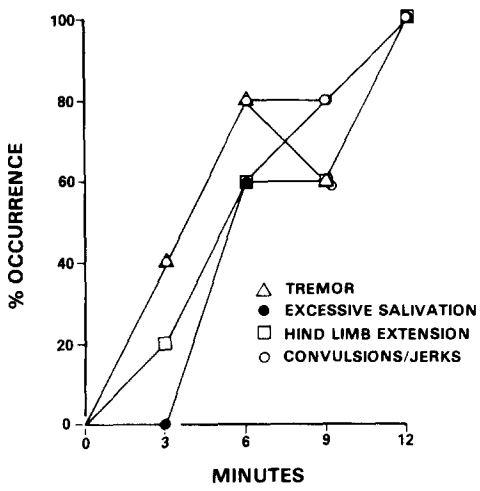

FIG. 3. The frequency of occurrence of toxic symptoms produced by soman (200 μg/kg) as a function of time after injection. N=6–8 per group. There was a significant change in symptom frequency with time for each group ($p<0.05$). There was no further change from the 12 min frequency values during the remaining 18 min of observation.

inhibition of soman's toxic symptoms (Table 2). The higher (0.5 mg/kg) dose of clonidine was equivalent in this respect to 6 mg/kg atropine. Combination of the lower (0.2 mg/kg) dose of clonidine with atropine resulted in a significantly more effective protective regimen than clonidine alone. Similar results were obtained for soman-induced chewing; however, soman inhibition of the three normal stereotyped behaviors (sniffing, locomotor activity and rearing) was only partially reversed by atropine and clonidine (Table 3). Thus, it was easier to inhibit the expression of soman-elicited behaviors than to prevent soman's depression of normal behaviors. While the combination regimen in most instances was more effective in terms of absolute value than atropine or clonidine alone, the difference between the combination regimen and atropine alone values did not reach the level of significance.

DISCUSSION

The protective effect of clonidine in soman poisoning in the rat is consistent with our earlier demonstration of protection in the mouse [5]. Employing the rat model, we were able to use established behavioral techniques to assess both soman-induced behavior as well as soman-induced suppression of normal stereotyped behavior. In both cases, clonidine reversed the effects of highly toxic doses of soman and enhanced the known protective actions of the classical antidote, atropine. Atropine-mediated protection from soman lethality and behavioral toxicity probably reflects its reversible inhibition of postsynaptic muscarinic receptors. In contrast, clonidine-mediated protection appears to involve at least three separate mechanisms: (1) a reversible inhibition of acetylcholine release from nerve terminals [2–4], (2) a reversible inhibition of AChE activity [1–5] and (3) a reversible inhibition of muscarinic receptors [5]. The effect of clonidine on acetylcholine metabolism in brain is mediated by presynaptic alpha$_2$-adrenergic receptors [3].

The ability of clonidine to suppress the behavioral changes elicited by soman in the rat is especially important in

TABLE 2

EFFECT OF ATROPINE AND CLONIDINE ON THE TOXIC SYMPTOMS PRODUCED BY SOMAN

Pretreatment (dose, mg/kg)	Percent Occurrence				
	Tremor	Excessive Salivation	Hind Limb Extension	Convulsions/ Jerks	N
Saline	100	81	45	91	11
Atropine (6)	30*	0*†	0*†	30*	10
Clonidine (0.2)	57*	57	43	57	7
Clonidine (0.5)	0*	0*	0*	0*	6
Atropine (6) + Clonidine (0.2)	0*†	0*†	0*	0*†	6

Rats were pretreated with protective agent for 30 min prior to soman (0.16 mg/kg). Each animal was observed until death or 30 min. The values refer to observations made 18 min post-soman.

*=Significantly different ($p<0.05$) compared to saline controls.

†=Significantly different compared to clonidine (0.2) value.

TABLE 3

EFFECT OF ATROPINE AND CLONIDINE ON THE STEREOTYPED BEHAVIORS INHIBITED OR PRODUCED BY SOMAN

Pretreatment (dose, mg/kg)	Percent Occurrence				
	Chewing	Sniffing	Locomotor Activity	Rearing	N
Saline	100	0	0	0	11
Atropine (6)	0*†	50*†	50*†	40*	10
Clonidine (0.2)	86	0	0	14	7
Clonidine (0.5)	0*	17*	66*	66*	6
Atropine (6) + Clonidine (0.2)	0*†	66*†	83*†	50*	6

Rats were pretreated with protective agent 30 min prior to soman (0.16 mg/kg). Each animal was observed until death or 30 min. The values refer to observations made 18 min post-soman.

*=Significantly different ($p<0.05$) compared to saline controls.

†=Significantly different compared to clonidine (0.2) value.

light of recent studies examining the ability of soman to induce seizures in experimental animals. Glenn *et al.* [6] suggested that the appearance of seizure activity following cumulative soman administration may actually be the neurological basis for the expression of several of the accompanying autonomic and behavioral signs. A direct correlation between the appearance of behavioral signs following soman administration and epileptiform activity measured in frontal cortex of freely-behaving rats was noted. Our results are consistent with this view insofar as clonidine is extremely potent at preventing both soman-induced autonomic and behavioral changes, and overt convulsive activity (e.g., straub tail and convulsions/jerks).

Our experiments in both mice and rats clearly indicate that the protective actions of clonidine and atropine are synergistic. This synergism may reflect the fact that each drug affects different aspects of the synaptic transmission process. Furthermore, the well known ability of atropine to enhance the release of acetylcholine (possibly the result of blockade of inhibitory presynaptic auto-receptors) [7], an action which might limit atropine's inhibitory effect, is reduced by clonidine. Nevertheless, these interactions are too complex to be accounted for by such a mechanism: While clonidine enhances the protective actions of atropine in soman toxicity, our recent studies have demonstrated that it also antagonizes some of the stereotyped behaviors elicited by atropine in rats [8]. The ability of clonidine to antagonize the behavioral toxicity induced by both a cholinergic stimulant (soman) and a cholinergic antagonist (atropine) may seem paradoxical. However, several factors may contribute to the ability of clonidine to enhance the protective action of atropine while reducing some of its behavioral side effects. For one thing, the ability of clonidine to inhibit the turnover rate of brain acetylcholine is not global. In fact cholinergic systems in some brain regions, including the striatum and hippocampus, are resistant to clonidine's inhibitory effect [2]. Protection against soman-induced lethality may involve brain areas which mediate both the autonomic and respira-

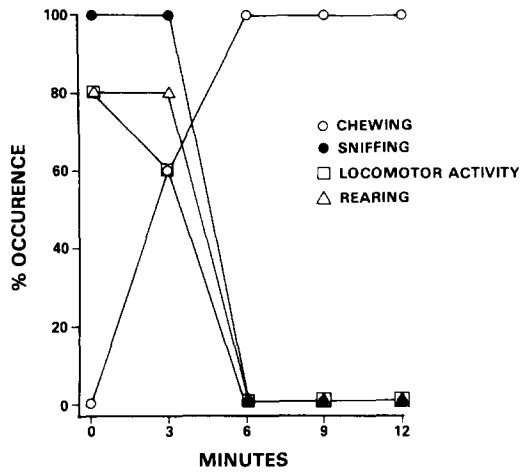

FIG. 4. The frequency of occurrence of behaviors influenced by soman (200 μg/kg) as a function of time after injection. There was a significant change in the behavior frequency with time for each group ($p<0.05$). There was no further change from the 12 min frequency during the remaining 18 min of observation.

tory changes induced by soman, such as the hypothalamus and medulla, both of which contain a high density of clonidine binding sites [10]. The cortex, a potential site for soman-induced seizure activity, is also well endowed with clonidine receptors. Atropine-induced stereotypy, on the other hand, may be mediated at the level of the striatum or hippocampus. Clonidine's ability to inhibit the expression of atropine-induced stereotypy may be related to stimulation or inhibition of non-cholinergic systems in these brain areas. Thus, while clonidine does not directly affect cholinergic neurons in the striatum or hippocampus, atropine can exert marked influences through its ability to inhibit muscarinic receptors in these areas. It is also possible that clonidine indirectly effects the expression of atropine-induced behavior by modulating the action of non-cholinergic fibers projecting to these areas.

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