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Toxic Interactions Between Repeated Soman and Chronic Pyridostigmine in Rodents[1,2,3]

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KERENYI, S. Z., M. R. MURPHY AND S. L. HARTGRAVES. *Toxic interactions between repeated soman and chronic pyridostigmine in rodents*. PHARMACOL BIOCHEM BEHAV **37**(2) 267–271, 1990.—These experiments examined the interactions of pyridostigmine, a reversible, peripherally acting anticholinesterase, with soman, an irreversible, peripherally and centrally acting anticholinesterase. Lethality, weight change, symptoms, and serum cholinesterase inhibition were determined following five daily injections of soman in rodents implanted with osmotic pumps containing two concentrations of pyridostigmine or vehicle. Concurrent exposure to both anticholinesterases had no effect on any measure at pyridostigmine-induced cholinesterase inhibition levels of 35% or 70% compared to controls. These results emphasize the safety of pyridostigmine as a pretreatment against organophosphate toxicity.

Anticholinesterase Soman Pyridostigmine Lethality

THE carbamate pyridostigmine (PYR), a peripherally acting reversible cholinesterase (ChE) inhibitor, has been used for many years in the treatment of a variety of disorders, including myasthenia gravis, as well as in the therapy of accidental drug overdose. Recently, the U.S. Military has fielded PYR to be used in repeated doses as a pretreatment for possible organophosphate (OP) exposure. The protective action of PYR (which requires postexposure therapy with anticholinergic and oximes for maximal effect) has been attributed to its ability to bind with a critical pool of acetylcholinesterase (AChE) and thus shield the AChE from attack by the irreversible OP. PYR may also afford protection by directly influencing membrane sensitivity in the periphery (13).

Although PYR (plus therapeutic drugs) has been shown to significantly protect against the lethal effects of the irreversible anticholinesterase (anti-ChE) soman (4, 7, 16), its effects on chronic exposure to OPs are less known or predictable. Of particular concern is the possibility that the total ChE inhibition, caused by both PYR and the OP, may eventually leave the individual so low in AChE that any additional inhibition, by either PYR or the OP, would be debilitating or life threatening. Documented cases exist of crop dusters and insecticide loaders who, after chronic exposure to low levels of organophosphorous insecticides and exhibiting no symptoms, had severe reactions to a subsequent acute exposure (5,15). From the perspective of the use of PYR as a pretreatment drug, the practical question is: Should PYR continue to be administered in the presence of low-dose chronic or repeated exposure to OPs?

In studies conducted on the effects of PYR pretreatment on response to soman, neither a single injection (11) nor 10 daily injections of PYR affected the lethality of acute exposure to soman (E. Grauer, personal communication). In contrast, Shiloff and Clement (14) reported continuous administration of PYR that caused 73% inhibition of whole blood ChE did potentiate the toxicity of acute soman exposure (the LD_{50} was lowered from 104 μg/kg to 82 μg/kg in rats); however, they reported no effects of chronic PYR that caused ChE inhibition of 65% or 25%.

The experiments reported here examined the effects on symptoms and LD_{50} of simultaneous exposure to chronic PYR and daily repeated soman over the course of 5 days.

GENERAL METHOD

Subjects

Male Sprague-Dawley rats (350 ± 50 g; Charles River, Por-

[1]Soman, >97% purity, was supplied by United States Army Chemical Research Development and Engineering Center.

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[3]The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council.

tage, MI) were quarantined and acclimatized in our vivarium for 10 days prior to use in these experiments. The animals were housed 2 to 3 per polycarbonate cage and were provided a standard laboratory diet and water ad lib before and after drug administration. The animal quarters were climate-controlled (22 ± 2°C) with a 12-hr light/dark cycle (0600–1800).

Pump Implantation

The Alzet® osmotic minipump (Model 2001) was used in this study to provide a stable level of drug in the animals for 7 days without the stress of multiple injections. Pumps were filled with a specific concentration of pyridostigmine bromide (Hoffmann-La Roche Co.) in a vehicle of acetic acid-sodium hydroxide, pH 5. The osmotic minipumps were presoaked in 37°C water to ensure uniform drug flow by alleviating the temperature gradient between the animal and pump (8). The rats were anesthetized with ketamine (100 mg/kg, SC) and a small (1.5 cm) incision was made in the skin below the back of the neck between the scapulae. The 7-day minipumps were implanted SC with the delivery-portal end first and the incision was closed with a wound clip.

Cholinesterase Poisoning Observations

All animals were weighed each day before soman injections and were monitored for ChE poisoning symptoms for 1 hr after the injections. The check sheet used to record weights and symptoms included the presence of salivation as well as the motor symptoms fasciculation, tremor, convulsion, and lying prostrate. Deaths were recorded 1 hr and 24 hr after each injection. Each motor symptom observed was given a score of 1 point with death awarded the maximum score of 5. Therefore, a mean symptom rating was established for each group (High PYR, Low PYR, or Control) each day after soman injection by averaging all the animals' symptom scores (from 1 to 5) across each group.

Cholinesterase Assay

The animals were decapitated on the 7th day after pump implantation and trunk blood was collected. The blood was centrifuged and serum was sampled to determine ChE activity using an enzyme reaction assay with acetylthiocholine as the substrate for AChE, modified from the method of Ellman *et al.* (6).

EXPERIMENT 1: EFFECTS OF CHRONIC PYRIDOSTIGMINE ON SERUM CHOLINESTERASE

Procedures

To select doses of PYR for Experiment 2, a dose-response curve for the effects of chronic PYR on serum ChE was generated using Alzet® minipump release rates of 0.0 (i.e., vehicle only), 0.5, 2.0, 8.0, and 16.0 mg/kg/day. Serum ChE was measured on the 7th day after pump implantation.

RESULTS

The effects of chronic PYR on percent inhibition (relative to vehicle infused controls) of serum ChE are shown in Fig. 1. Based on linear interpolation of the results of Experiment 1, two doses of PYR (1.0 and 10.0 mg/kg/day) were chosen for Experiment 2. The lower dose was selected to inhibit ChE at 40% while the higher dose was selected to inhibit ChE at 80%. The U.S. Military has established the PYR pretreatment target level at 30 ± 10% erythrocyte ChE inhibition.

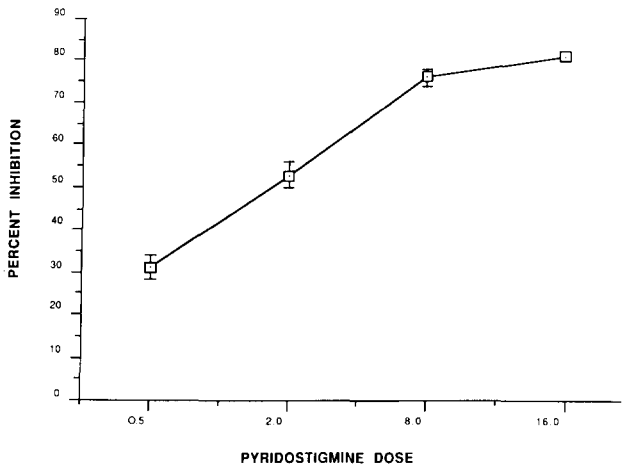

FIG. 1. The effect of chronic pyridostigmine in mg/kg/day on serum ChE inhibition relative to vehicle-infused controls.

EXPERIMENT 2: PYRIDOSTIGMINE AND SOMAN INTERACTIONS

Procedures

The interactions between chronic PYR and daily repeated soman were assessed. Three groups of rats were implanted with Alzet 7-day minipumps designed to release 0.0, 1.0 or 10.0 mg/kg/day PYR and, thereby, cause chronic serum ChE inhibition of 0.0%, 40%, 80%, respectively (n = 34 per group). Starting 3 days after the implantation, and continuing for 5 days, members of each group received daily subcutaneous injections of soman (1 ml per kg of subject body weight) diluted with 0.9% saline, as follows: 32 μg/kg/day (n = 10); 39 μg/kg/day (n = 7); 48 μg/kg/day (n = 10); or 59 μg/kg/day (n = 7). Body weight was recorded daily prior to the injection period. Symptoms of ChE poisoning, including death, were recorded daily 1 hr after soman injections. One hour after the 5th injection, surviving animals

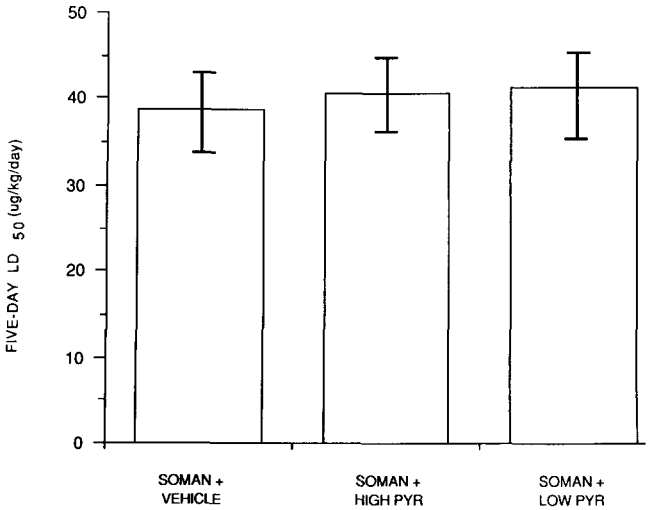

FIG. 2. The five-day soman LD_{50} with or without chronic pyridostigmine pretreatment at a high (10 mg/kg/day) or low (1.0 mg/kg/day) dose.

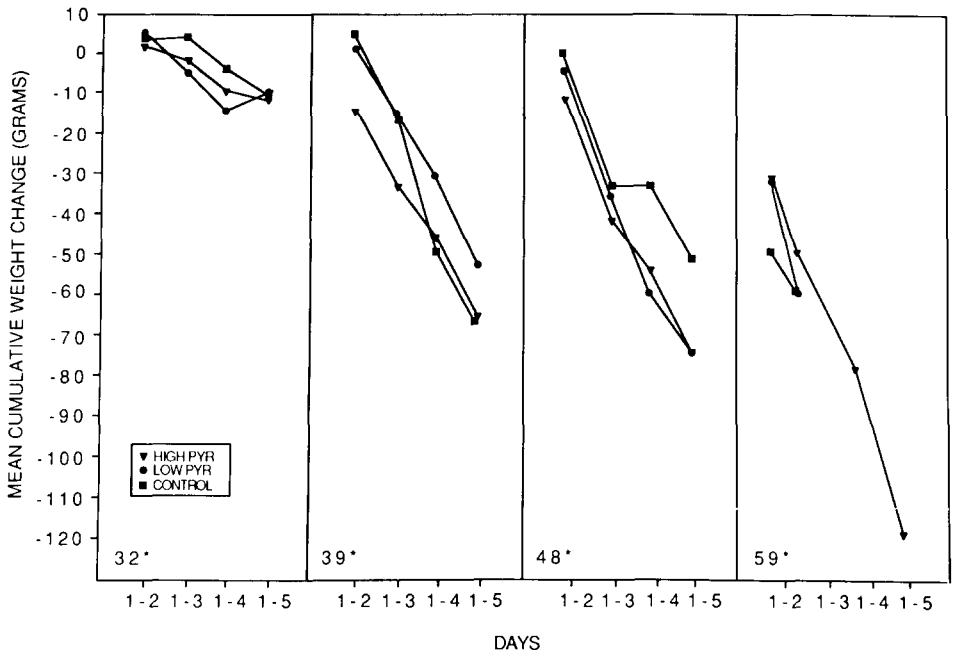

FIG. 3. Chronic PYR: effect on soman-induced changes in body weight. Repeated soman dose, *, is in μg/kg/day.

were decapitated for serum ChE assay. An LD_{50} for daily repeated soman was calculated using the method of probits.

To determine a standard against which the LD_{50} of repeated soman doses could be compared, an acute LD_{50} was determined.

To validate the dose rates used, the effects of 0.0, 1.0, and 10.0 mg/kg/day PYR on serum ChE activity were measured 7 days after pump implantation in separate groups of rats; an untreated control group was also included (n = 7 per group).

RESULTS

The acute LD_{50} of soman was estimated to be 121.5 μg/kg (95% confidence interval: 97.2–151.8). Since serum ChE activity did not significantly differ between untreated and implanted

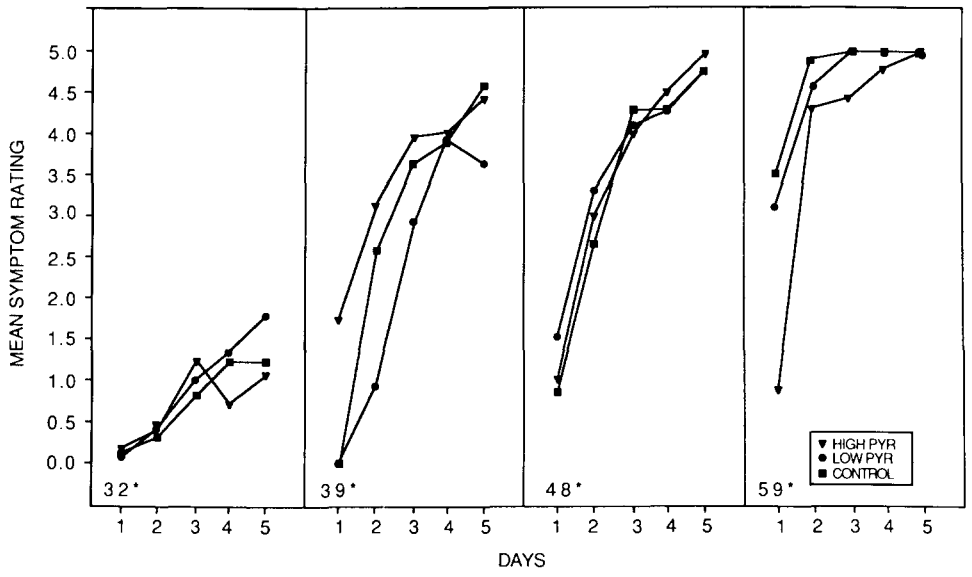

FIG. 4. Chronic PYR: effects on soman-induced motor symptoms. Soman dose, *, is in μg/kg/day.

TABLE 1

PERCENT INHIBITION OF SERUM ChE IN SURVIVORS PRETREATED WITH PYRIDOSTIGMINE AT A HIGH OR LOW DOSE, ONE HOUR AFTER 5TH SOMAN INJECTION, AS COMPARED TO CONTROLS (N = 14)

		High-Dose Pyridostigmine (10.0 mg/kg/day)	Low-Dose Pyridostigmine (1.0 mg/kg/day)	Vehicle (Control)
Soman μg/kg/day (daily injection)	32	85.7 (10/10)	83.9 (9/10)	86.0 (9/10)
	39	87.2 (4/7)	87.4 (5/7)	91.0 (2/7)
	48	89.7 (1/10)	84.4 (2/10)	89.1 (2/10)
	59	— (0/7)	— (0/7)	— (0/7)

Number of surviving subjects out of total number in parentheses.

controls, the data from these groups were combined. Compared to these controls, ChE in animals receiving 1.0 or 10.0 mg/kg/day PYR was inhibited by 35% and 70% respectively.

Neither rate of PYR release had any significant effect (positive or negative) on response to daily repeated soman either on LD_{50} (Fig. 2), total weight loss (Fig. 3), symptoms (Fig. 4), or serum ChE of survivors (Table 1). An independent ChE measure, conducted on animals exposed acutely to soman, revealed that the ChE inhibition plateau is reached after the first injection: a single soman dose of 34.5 μg/kg (n = 8) produced 83% serum ChE inhibition as compared to controls (n = 14), while a dose of 43.0 μg/kg (n = 8) inhibited ChE by 87%.

GENERAL DISCUSSION

Our results should be compared with those of Shiloff and Clement (14), who showed that chronic pretreatment of rats with PYR (which inhibited whole blood AChE by 73%) sensitized the animals to the toxic effects of a subsequent acute dose of soman. We saw no such sensitization during chronic soman exposure at PYR-induced blood serum ChE inhibition levels of 70%. The explanation for the differences between our results and those of Shiloff and Clement may lie in the PYR-induced ChE inhibition levels. While our highest serum inhibition levels of 70% approached their highest whole blood inhibitions levels of 73%, the same dose of anti-ChE usually produces different ChE inhibition in different blood components (2, 3, 9, 12). Thus, we expect that the differences in PYR inhibition of ChE between the studies may have been much greater than at first apparent, with the high-dose PYR animals in the Shiloff and Clement study exhibiting much greater ChE inhibition than our high-dose PYR subjects. However, more important than the differences between these studies is the result that neither study found any negative interaction between either acute or repeated soman and PYR at lower pretreatment levels (30%–70% serum ChE inhibition and 25% to 65% whole blood ChE inhibition).

The results of this study indicate that, in rats, effects of simultaneous chronic exposure to PYR and low-dose soman are no different from chronic exposure to soman alone. The presence of PYR neither protected against nor exacerbated soman-induced lethality, symptoms, or weight loss. This finding is significant because it suggests that the use of the pretreatment carbamate PYR as protection against soman poisoning will not potentiate subtle symptoms of low dose exposure and can safely be continued. However, the failure to find any protection from the peripherally acting PYR suggests that more centrally acting drugs, such as physostigmine, should be considered as pretreatments for possible OP poisoning.

This research was conducted using the rat, a species that, because of high levels of serum carboxylesterase, is a great deal less sensitive to soman than are primates, including man (10). For this reason, this research has been repeated using a primate model. The results are reported in a separate paper (1).

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