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The Effects of Pyridostigmine Bromide and Permethrin, Alone or in Combination, on Response Acquisition in Male and Female Rats

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VAN HAAREN, F., B. CODY, J. B. HOY, J. L. KARLIX, C. SCHMIDT, I. R. TEBBETT AND D. WIELBO. *The effects of pyridostigmine bromide and permethrin, alone or in combination, on response acquisition in male and female rats.* PHARMACOL BIOCHEM BEHAV **66**(4) 739–746, 2000.—It has been hypothesized that concurrent exposure to pyridostigmine bromide and permethrin may have contributed to the development of neurocognitive symptoms in Gulf War veterans. The present experiment was designed to investigate the effects of pyridostigmine bromide and permethrin alone, or in combination, on the acquisition of a novel response, one measure of normal cognitive functioning. Male and female Sprague–Dawley rats were treated with pyridostigmine bromide (1.5 mg/kg/day, by gavage in a volume of 5 ml/kg) or its vehicle for 7 consecutive days. They then also received an intraperitoneal injection of permethrin (0, 15, or 60 mg/kg) before they were exposed to an experimental session during which they could earn food by pressing a lever in an operant chamber. Serum permethrin levels increased as a function of its dose, and were higher in rats treated with pyridostigmine bromide. Sex differences were observed as permethrin levels were higher in female rats than in male rats following the highest dose. Pyridostigmine bromide delayed response acquisition in male and female rats, and resulted in higher response rates on the inactive lever in female rats than in male rats. Although permethrin levels were higher in subjects treated with pyridostigmine bromide than in those treated with vehicle, there were no differences in the behavioral effects of permethrin. Whether or not these behavioral effects of pyridostigmine bromide are of central or peripheral origin will need to be determined in future studies, as its effects on motor activity and/or gastro-intestinal motility may have affected response acquisition. © 2000 Elsevier Science Inc.

Gulf War Illness Pyridostigmine bromide Permethrin Cholinesterase inhibition Synergism Learning Response acquisition Lever press male and female rats

CONCURRENT exposure to pyridostigmine bromide (PB), a carbamate cholinesterase inhibitor, and the pyrethroid insecticide permethrin (PERM) may have contributed to the development of a syndrome that appears to have afflicted military personnel who served during the Gulf War (5,9–11,14,26).

PB is a quartenary ammonium compound that inhibits the hydrolysis of acetylcholine (ACh) by competitive reversible binding to acetylcholinesterase (AChE). It has been suggested that PB may decrease nerve gas toxicity by occupying AChE binding sites (33). Reportedly, PB was taken prophylactically during the Gulf War (three × 30 mg/day/70 kg for up to 21 days) when there was a high risk of nerve gas exposure (14).

The synthetic pyrethroids, of which PERM is one, are widely used insecticides that have been divided into two classes according to their chemical properties and toxicity symptoms (32). Toxic exposure to PERM, a Type I compound, is evidenced by aggressive sparring, hypersensitivity to external stimuli, whole body tremor and prostration in experimental animals [cf. (19)]. These symptoms are thought to originate in the central nervous system, as they have been

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shown to correlate with the concentration of unmetabolized pyrethroid in brain tissue (8). PERM was used to impregnate battle-dress uniforms in the field during the Gulf War, but the extent of its usage is not known.

Some of the behavioral effects of small doses of PB and PERM have been documented before. Wolthuis and Vanwersch (33) determined in rats that PB decreased two-way shuttlebox avoidance efficiency, decreased open-field locomotor activity and produced a dose-dependent decrease in the number of correct steps in a hurdle-stepping task, at less than 10% of the intraperitoneal LD_{50}. In other studies, Liu and his colleagues (16,17,22) tested the effects of PB on schedule-controlled behavior. They observed that low doses of PB (3–12 mg/kg, by gavage) which did not produce any overt signs of toxicity, decreased fixed-ratio (FR) 30 response rates, whereas higher doses (30 and 40 mg/kg) completely eliminated responding. It has recently been reported that PB dose dependently decreased locomotor activity in male and female rats, but that female rats were affected by lower doses than males (13). In another experiment, we showed that acute and repeated PB administration delayed response acquisition, when reinforcers were either presented immediately after a response or following a short delay (30).

Small doses of PERM that did not produce any overt signs of neurotoxicity have been shown to dose dependently decrease responding maintained by a variable-interval 20-s (VI 20-s) schedule of reinforcement (3). When rats were trained to respond on a variable-ratio 25 (VR 25) schedule (23), the highest dose of PERM (60 mg/kg, IP) significantly decreased response rates. Peele and Crofton (21) exposed male Long–Evans hooded rats to a four-component multiple (VI 10-s, VI 30-s, VI 90-s, VI 270-s) schedule of food reinforcement, and tested different doses of PERM (vehicle, 100, 200, 300, and 400 mg/kg), which they administered per os, 90 min prior to the start of the session. Response rates decreased dose dependently, and the oral ED_{50} was established at 350 mg/kg in this experiment.

It has been reported that the neurotoxicological effects of PB and PERM combinations may exceed the effects of the individual compounds. McCain, Lee, Johnson, Whaley, Ferguson, Beall, and Leach (18) assessed the LD_{50} of PB and PERM either alone, or in combination, and reported that different doses of PB in combination with PERM killed more male laboratory rats than would have been expected if the effects of the compounds had merely been additive. Similarly, Abou-Donia and his colleagues have recently shown in hens that the behavioral and neurotoxicological effects of combined treatment with PB and PERM exceeded those observed after administration of the individual compounds (1). These investigators suggested that the effects of the compound combinations might be a function of the fact that PERM is more likely to penetrate the central nervous system when PB is present in the circulation.

It has been suggested that the intellectual and neurocognitive functioning in veterans presenting Gulf War Syndrome may have been compromised by concurrent exposure to PB and PERM, or other compounds employed in the war theater (12). The present experiment is one in a series of studies designed to assess the effects of small, but behaviorally active doses of PB and PERM, alone or in combination, on different behavioral end points, in this case the acquisition of a novel response (learning). In these experiments, naive, food-restricted subjects are given the opportunity to obtain food by pressing one of two levers in an experimental chamber. Previous experiments have shown that untreated control subjects quickly learn to press the lever associated with reinforcement presentation (15,24,25,27), but that acute and repeated PB administration delayed response acquisition (30). It was hypothesized that concurrent PB and PERM administration might further delay the acquisition of a novel response.

Different groups of male and female subjects were treated with an amount of PB approximately equal to the Gulf War dose (1.5 mg/kg/day for 7 days by gavage), or they were treated with distilled water. They then received an intraperitoneal injection of PERM (vehicle, 15 or 60 mg/kg) before an experimental session during which lever presses (novel response) were followed by food presentation. The doses of PERM were chosen to reflect those that had been behaviorally active in other experiments. Whether or not these doses approximate potential Gulf War exposure levels has not yet been determined. The lever press response was not shaped in any way, but left to emerge spontaneously. Male and female rats participated in this experiment because it has been shown that the behavioral consequences of PB and PERM administration, just like those of other substances, may be affected by sex hormones (2,13,20,28,29,31).

METHOD

Subjects

Forty-eight male and 48 female Sprague–Dawley rats were obtained from a commercial supplier (Zivic-Miller, Zelienople, PA) when they weighed approximately 225–250 g. They were housed in same-sex pairs under a reversed light–dark cycle (lights on 1800 h), and allowed free food and water for 1 week. Access to food was then limited for the remainder of the experiment (16 g/day per male rat and 12 g/day per female rat, offered at 1700 h), while tap water remained continuously available.

Apparatus

The experiment was conducted in six identical Coulbourn Instruments modular rodent operant-conditioning chambers, which were 25 cm wide, 30 cm long, and 29 cm high (Allentown, PA). The sidewalls of each chamber were made of Plexiglas; the back wall and the intelligence panel were made of stainless steel. The floor consisted of 16 rods, spaced 2 cm apart (center to center). Two retractable rodent levers were located symmetrically to the side of the pellet tray, 6.3 cm from the floor of each chamber. When extended, the levers protruded 1.8 cm from the intelligence panel and required a force of more than 0.20 N to be operated. There were three stimulus lights directly above each lever, and a house light was located 3 cm from the ceiling in the middle of the intelligence panel. The pellet tray was illuminated by a white light bulb during the delivery of a food pellet (Noyes, 45 mg purified rodent formula). Each experimental chamber was housed in an individual sound-attenuating, ventilated cabinet. The chambers were connected to an IBM-PC compatible microcomputer (GatorByte, Gainesville, FL) through a LabLinc interface (Coulbourn Instruments LPC, Allentown, PA) located in the experimental room itself. Experimental contingencies and data acquisition procedures were programmed in L2T2 (Coulbourn Instruments LPC, Allentown, PA).

Procedure

Magazine training. The subjects were placed in the darkened operant chamber from which the levers had been retracted 5 min before the start of the session. At the beginning

of the magazine training session, the house light was illuminated. Pellet delivery, which was accompanied by brief illumination of the light in the pellet tray, was then programmed to occur once every 60 s, on average, on a random-time (RT) schedule until 60 pellets had been presented. Most subjects had retrieved all pellets from the tray at the end of the session; the few subjects who had not, received an additional training session. Magazine training was completed before any drugs were administered.

Response acquisition session. Subjects were put into the dark operant chamber 5 min before the beginning of the session at 1600 h, to include the final 2 h of the subjects' dark period. Experimental sessions had to be arranged in this manner so as not to interfere with other experiments that were being conducted during the regular daytime hours. The house light was illuminated at the beginning of the session and the two levers were extended into the experimental chamber. During the response acquisition session, each press on the left (operative) lever immediately resulted in the presentation of a food pellet, while a press on the right (nonoperative) lever was recorded but did not have any scheduled consequences. The experiment was terminated after 8 h, and the subjects were immediately removed from the experimental chamber. All response acquisition sessions were conducted on consecutive days.

Drug administration. Half of the subjects received distilled water once a day for 6 consecutive days at 1600 h. On day 7, some subjects received distilled water 15 min prior to the administration of PERM vehicle, which occurred 15 min prior to the beginning of the experimental session ($n = 7$ male rats, $n = 5$ female rats). Other subjects received distilled water followed by 15 mg/kg PERM ($n = 8$ male rats, $n = 4$ female rats), while the remaining subjects received distilled water followed by 60 mg/kg PERM ($n = 8$ male rats, $n = 8$ female rats). The other half of the subjects received 1.5 mg/kg PB once a day for 6 consecutive days. On day 7, some subjects received 1.5 mg/kg PB 15 min prior to the administration of PERM vehicle, which occurred 15 min before the start of the experimental session ($n = 7$ male rats, $n = 8$ female rats). Other subjects received 1.5 mg/kg PB and 15 mg/kg PERM ($n = 8$ male rats, $n = 8$ female rats) or 1.5 mg/kg PB and 60 mg/kg PERM ($n = 7$ male rats, $n = 6$ female rats). PB and its vehicle were administered by gavage in a volume of five ml/kg, PERM and its vehicle were administered IP in a volume of two ml/kg. All experimental groups had been designed to consist of eight subjects each, but data from some of the subjects had to be excluded from the final analyses due to equipment malfunction during the course of some experimental sessions. The day following the response acquisition session all subjects received the same drug treatment that they had also received the day before. This allowed us to evaluate PB and PERM serum levels following a pretreatment time identical to that of the behavioral experiments. Vaginal smears were obtained from female rats before both the operant acquisition session and the next day. These samples were collected to allow us to analyze behavioral and physiological variables in the context of the stage of estrus cycle at the time of testing.

Drug Preparation

Pyridostigmine bromide (PB) was obtained from Sigma Chemical Co. (St. Louis, MO) and dissolved in distilled water. Technical grade permethrin (PERM [3-phenoxyphenyl) methyl(+)-*cis,trans*-3-(2,2-dichloroethenyl)-2,2-dimethylchloropropanecarboxylate], minimum 35% (±*cis*, and maximum 65% (±)*trans*) was obtained from Coulston Products (Easton, PA, procured via Dr. W. McCain, Aberdeen Proving Grounds, MD) and prepared in a vehicle of equal volumes of Emulphor and 95% ethanol (total volume of 0.2 ml/10 mg of PERM). This mixture was diluted with 0.9% physiological saline to the desired concentrations.

Serum Preparation

Each rat was placed in a jar containing a paper towel saturated with Metofane (Methoxyflurane) for less than 1 min. The anesthetized animal was then quickly decapitated. Blood was collected in a 15-ml polystyrene culture test tube and allowed to coagulate on ice for 2 h. It was then centrifuged for 15–20 min at approximately 3000 revolutions per minute. The serum was drawn off the solid cell matter with a clean glass Pasteur pipette and placed in a 1.5-ml polystyrene microcentrifuge tube. It was then immediately placed in a freezer (at −20°C), where it was stored until analysis.

Neurochemical Analyses

Pyridostigmine bromide. The 0.5-ml serum sample was transferred to a stoppered tube and vortexed with 1 ml of 0.025 M potassium phosphate buffer at pH 3. This mixture was then applied to a Strong Cation Exchange column that had previously been conditioned under vacuum on a Vac Elut manifold (Analytichem) with methanol (2 ml), water (1 ml), and 0.25 M phosphate buffer (1 ml). After application of the sample, the column was air dried for approximately 30 s, and then washed with phosphate buffer (1 ml) and 0.1 M acetic acid. The column was again air dried for 30 s before eluting off the adsorbed drugs with ammoniacal methanol (3%, 2 ml). The final extract was evaporated to dryness under nitrogen and the residue reconstituted in 50 μl of methanol. A 20-μl aliquot of the extract was used for HPLC analysis. This analysis was performed using a Waters 510 pump to deliver solvent at 1 ml/min to a Hypersil 5um ODS column (25 cm × 4.5 mm i.d.) column. A Waters C18 Guard Pak precolumn was used to protect the analytical column. The Detector was a Waters 486 variable wavelength detector set at 272 nm with a Dell 486 data system and Millenium™ software. The mobile phase consisted of acetonitrile–0.1% triethylamine in water (adjusted to pH 3.2 with phosphoric acid 70:30). Quantitative analysis was achieved by comparison of peak areas with unextracted standards. Each determination was taken as the mean of three replicate injections. The calibration graph was produced over the range of 0.05–5 μg/ml.

Permethrin. A 200-mg Clean Screen solid phase extraction cartridge (sorbent type CSDAU, manufactured by Worldwide Monitoring) was conditioned with 2 ml acetone, 2 ml methanol, and 2 ml deionized water. A 0.5-ml volume of sample rat serum was transferred to the cartridge reservoir and allowed to percolate by gravity through the sorbent bed. The cartridge was washed with 2 ml deionized water, placed on a vacuum manifold, and dried under full vacuum for approximately 5 min. Permethrin was eluted from the cartridge with a 1-ml volume of acetone and collected in a graduated conical tube. A 10-μl volume of internal standard (40 ng/μl of US108, purchased from Ultra Scientific) was added to the tube, the final volume was adjusted to 1 ml, and the extract was transferred to a gas chromatograph vial for analysis.

The extracts were analyzed for permethrin using a Hewlett Packard 6890 gas chromatograph coupled to a Hewlett Packard 5973 mass selective detector operating in the electron im-

pact mode. The gas chromatograph was equipped with a 30-m HP-5MS column (250 μm diameter with a 0.25 μm film thickness) operated in the splitless mode at a flow rate of 0.8 ml/min. A 1 μl aliquot of the final extract was injected into the gas chromatograph, and the analytes were separated using the following temperature program. The inlet temperature was set at 275°C and the initial oven temperature was set at 40°C. The initial oven temperature was held for 4 min, and then ramped to 270°C at 10°C/min. The oven was held at 270°C for 5 min, then ramped to 300°C at 25°C/min. the final temperature was maintained for 6.8 min. Under these conditions, the retention times for *cis*-permethrin and *trans*-permethrin were 27.6 and 27.8 min, respectively. The detector was operated in the selected ion-monitoring mode with an electron impact voltage of 70 eV and an electron multiplier voltage of 1882 V. Both forms of permethrin were quantitated using ion 183 and confirmed using ions 163 and 165. The internal standard was quantitated using ion 188.

Statistical Analyses

The experimental design included SEX (male and female rats), two levels of repeated drug administration (PB or vehicle), three levels of acute pesticide administration (PERM vehicle, 15 mg/kg or 60 mg/kg) and TIME (repeated observations within the experimental session). PB levels and PERM levels were subjected to a three-way analysis of variance to analyze the effects of the subjects' gender (SEX), chronic PB administration (PB), and acute PERM administration (PERM). The number of responses at each hour of the experimental session was analyzed by analysis of variance, which included these same three factors and the factor time (repeated within subjects). Repeated-measures analysis of variance allowed us to assess the effects of the subjects' gender, chronic PB administration, acute perm administration, and time since the start of the session on response acquisition.

RESULTS

Figure 1 shows serum PERM levels for male and female rats treated with different doses of PERM in the presence or absence of PB. Serum levels of PERM increased as a function of PERM dose, $F(1, 43) = 52.68$, $p < 0.0001$, and were higher in rats treated with PB than in rats treated with distilled water only, $F(1, 43) = 4.79$, $p < 0.03$. There was a significant three-way interaction between SEX, PB, and PERM, $F(1, 43) = 21.17$, $p < 0.0001$. Post hoc analyses showed that serum PERM levels were higher in females than in males following 60 mg/kg PERM both in PB-treated and vehicle-treated subjects ($p < 0.0027$ and $p < 0.0001$, respectively). It should be noted that repeated administration of 1.5 mg/kg PB resulted in PB serum levels 30 min following the final administration of PB that were below the detection limit of our assay in all groups of subjects.

The behavioral results of the experiment are presented in Figs. 2, 3, and 4. They show the cumulative number of reinforced responses on the active lever observed in 10-min segments of the experimental session for individual male (Fig. 2) and female subjects (Fig. 3). The data for subjects treated with PB vehicle and the different doses of PERM are shown in the left-hand panels of Figs. 2 and 3, those for subjects repeatedly treated with 1.5 mg/kg PB and the different doses of PERM are shown in the right-hand panels. Open circles reflect the number of responses observed on the inactive lever during every hour of the experimental session.

Figure 4 presents these same data, but averaged over groups of subjects to allow for more direct comparisons of the data across drug conditions and gender. The data for male and female rats are shown in the left- and right-hand panels, respectively, the top panels show the effects of PERM in vehicle-treated subjects, the bottom panels those in PB-treated subjects. The filled symbols show cumulative lever presses in subjects treated with PB vehicle and PERM vehicle, i.e. essentially untreated subjects.

Control male and female rats treated with PB and PERM vehicles (upper left-hand panels in Figs. 2 and 3) quickly and consistently initiated responding on the operative lever from the onset of the experimental session. PB administration reduced the number of responses on the operative lever as a function of the time since the beginning of the session [PB × time, $F(7, 518) = 2.56$, $p < 0.0133$; time, $F(7, 518) = 38.90$, $p < 0.0001$; PB, $F(1, 72) = 3.52$, $p < 0.0648$] in male and female rats. Post hoc analyses showed a higher number of reinforced responses when subjects had been treated with distilled water than when they had been treated with PB during the first 2 h of the experimental session. Perm administration did not affect response acquisition. Sex differences were not observed in response acquisition on the active lever, but PB administration affected overall response rates (responses per minute) on the inactive lever in a sex-dependent manner [SEX × PB,

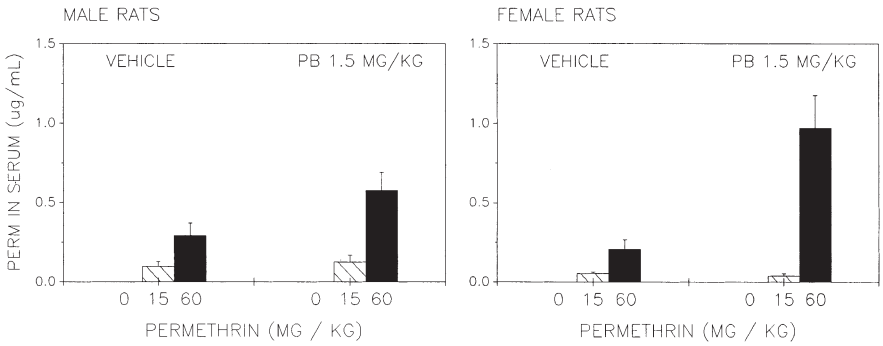

FIG. 1. Average serum permethrin levels (±1 SEM) in male rats (left panel) and female rats (right panel) treated with different doses of permethrin (0, 15, or 60 mg/kg) in the presence or absence of repeated administration of 1.5 mg/kg pyridostigmine bromide.

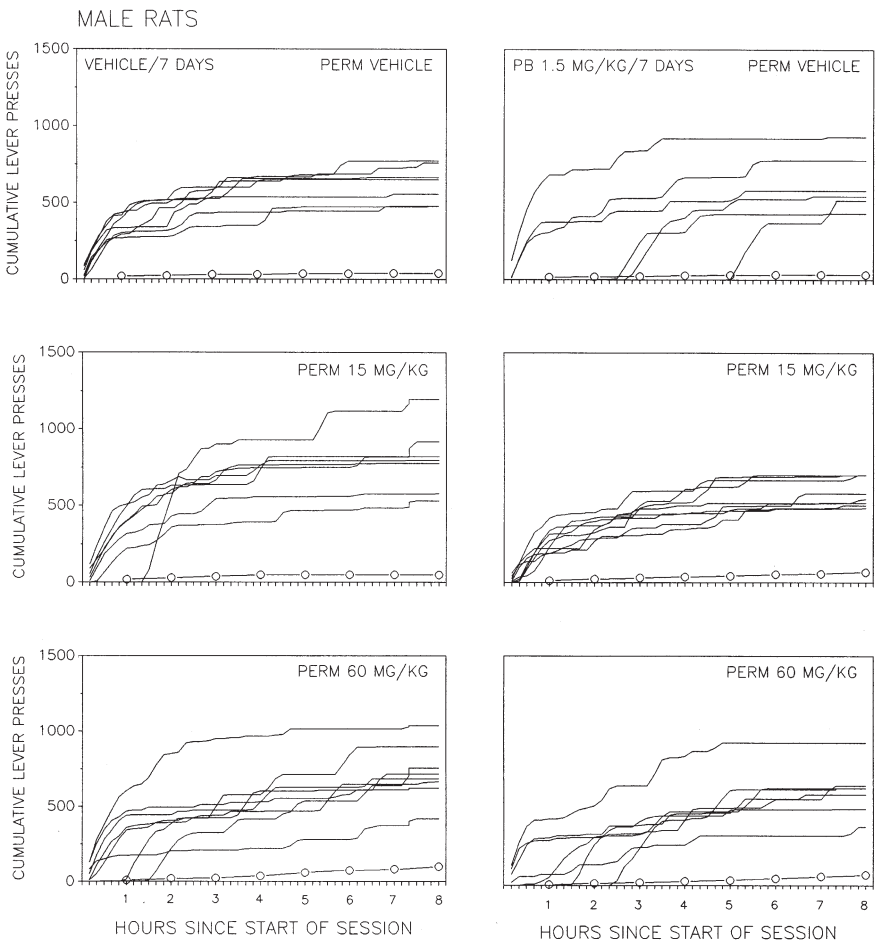

FIG. 2. The cumulative number of reinforced responses observed in 10 min segments of the experimental session for individual male rats. The data for subjects treated with pyridostigmine bromide vehicle and the different doses of permethrin are shown in the left-hand panels of the figure, those for subjects repeatedly treated with 1.5 mg/kg pyridostigmine bromide and the different doses of permethrin are shown in the right-hand panels of the figure. Open circles reflect the number of responses observed on the inactive lever during every hour of the experimental session.

$F(1, 72) = 5.50, p < 0.0217$]. There was no difference between males and females following the administration of PB vehicle, but females responded much more on the inactive lever than males following repeated PB administration ($p < 0.0096$). Analysis of vaginal smears did not reveal any obvious relationship between stage of estrus cycle at the time of testing and any of the behavioral and physiological measures.

DISCUSSION

The present experiment was designed to investigate to what extent repeated PB administration alone, or in combination with the acute administration of PERM would affect response acquisition in male and female rats. PB's repeated dose was chosen to mimic that of possible, short-term, Gulf War exposure (1.5 mg/kg PB by gavage in 5 ml/kg). The doses of PERM were similar to those previously shown to be behaviorally active, yet not toxic [e.g., (23)].

The experiment has yielded a number of interesting observations. Serum PERM levels increased as a function of its dose and, in the presence of repeated PB administration, they were higher in female rats than in male rats following the highest dose of PERM (60 mg/kg). These observations are interesting in the context of observations by others (1) that the behavioral and neurotoxicological effects of the combined treatment with PB and PERM exceeded those observed after the administration of the individual compounds. The results of the present experiment suggest that even very low, but repeatedly administered, doses of PB may not only affect PERM serum levels, but that they do so in a sex-dependent manner. These sex differences in the neurochemical interactions between PB and PERM warrant further scrutiny in future experiments. This especially in view of the fact that others have reported that PB actually may reduce PERM penetration into the brain (4). Analysis of vaginal smears obtained in this experiment in the context of PERM levels did not reveal any obvious correlations, but such should not be taken to indicate that repeated PB administration is without

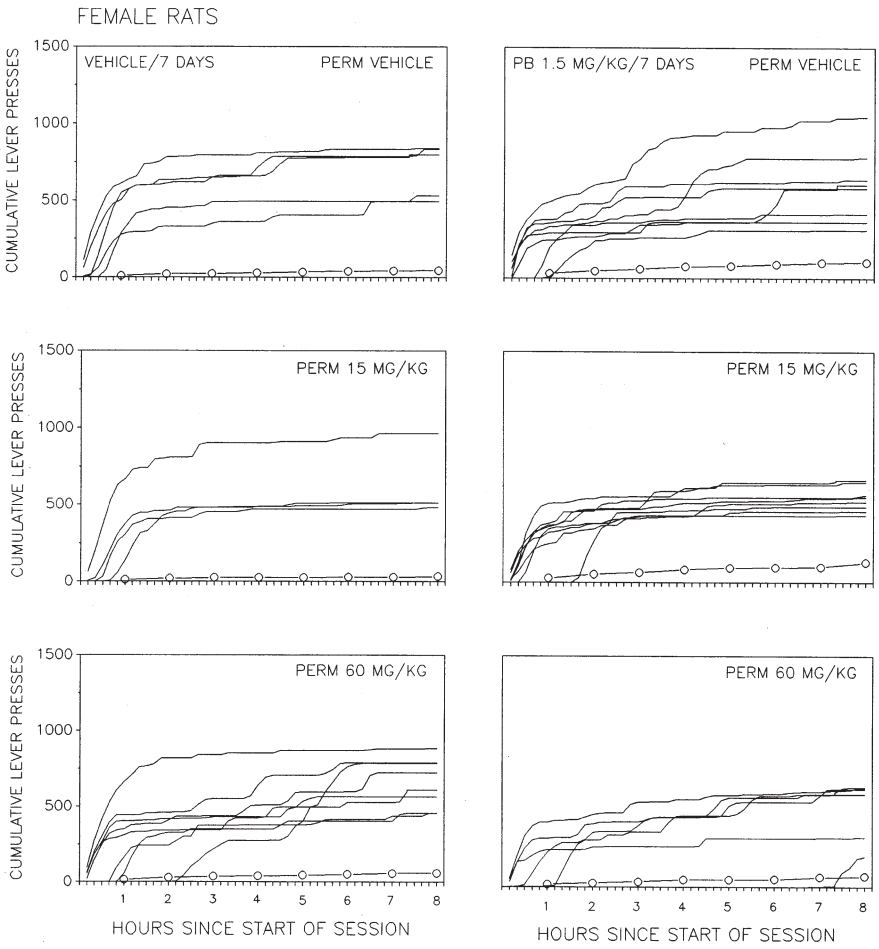

FIG. 3. The cumulative number of reinforced responses observed in 10-min segments of the experimental session for individual female rats. The data for subjects treated with pyridostigmine bromide vehicle and the different doses of permethrin are shown in the left-hand panels of the figure, those for subjects repeatedly treated with 1.5 mg/kg pyridostigmine bromide and the different doses of permethrin are shown in the right-hand panels of the figure. Open circles reflect the number of responses observed on the inactive lever during every hour of the experimental session.

gender-dependent effects (vide supra). Experiments in intact and gonadectomized male and female rats with and without hormone replacement (testosterone propionate, estradiol, and progesterone) should shed more light on these questions.

PB administration delayed the time at which responding was initiated, and decreased the number of reinforced responses in male and female rats. As such, these results confirm and extend those of another study in which it was shown that acute and repeated PB administration lowered the number of reinforced responses when response acquisition was examined under conditions of immediate and delayed reinforcement (30). PB administration resulted in higher response rates on the inactive lever in female rats than in male rats. PERM alone did not affect response acquisition in male and female rats. This observation is in contrast to those of other experiments in which it was shown that similar doses of PERM dose dependently decreased well-established schedule-controlled performance (3,21,23). It is interesting to note that there were no sex differences in response acquisition despite the fact that PB administration affected PERM levels more in female rats than in male rats. This is not to say that the sex differences in neurochemical parameters do not appear to have behavioral consequences. Other studies conducted in our laboratory, for instance, have shown that acute PB administration results in a sex-dependent decrease in locomotor activity (13). It should be noted that the repeated dose of PB was chosen to resemble that which may have been used frequently during the Gulf War. The results of the present experiment in conjunction with those of other studies (13,30) appear to indicate that the functional consequences of this low dose of PB should not be underestimated.

Did PB act on the central nervous system (CNS) or the peripheral nervous system (PNS)? It has been assumed that PB, as a quartenary carbamate, does not cross the blood–brain barrier (BBB), but a number of recent findings suggest that PB's effects may be centrally mediated. First, evidence has been presented to show that stress may make it easier for PB to penetrate the BBB. Friedman, Kaufer, Shemer, Hendler,

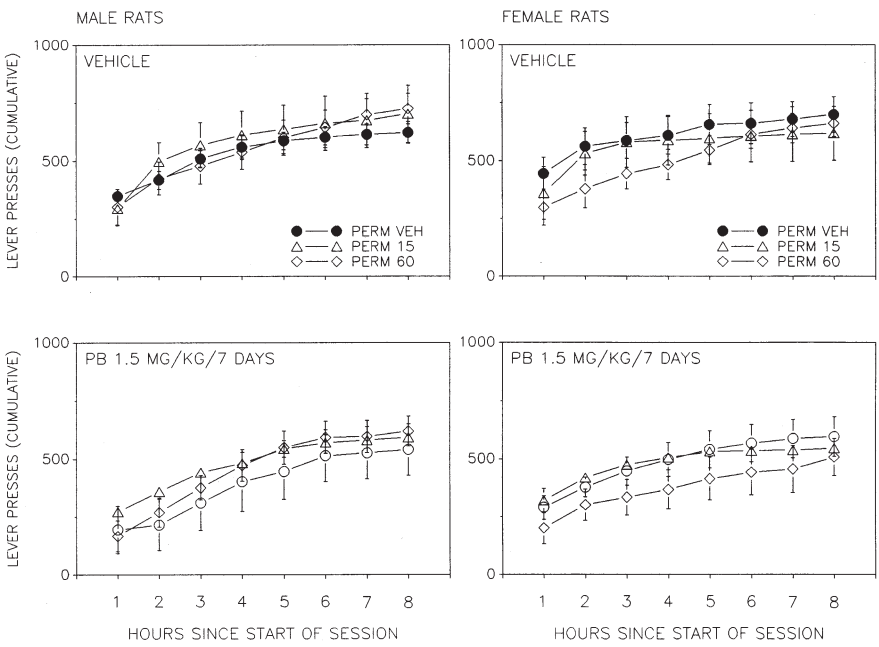

FIG. 4. The average cumulative number of reinforced responses (±1 SEM) in the different treatment groups at each hour of the experimental session. The data for male and female rats are shown in the left- and right-hand panels, respectively; the top panels show the effects of permethrin in vehicle-treated subjects; the bottom panels those in subjects treated with pyridostigmine bromide. Circles represent data from those subjects treated with the permethrin vehicle, while triangles and diamonds represent data from subjects treated with 15 and 60 mg/kg permethrin, respectively. The filled circles show cumulative lever presses in subjects treated with the pyridostigmine bromide vehicle and the permethrin vehicle, i.e., essentially untreated subjects.

Soreq, and Tur-Kaspa (7) have shown that swim stress reduced the dose of PB required to inhibit brain AchE activity by 50% to less than 1/100th of the dose in subjects that had not been stressed. It has also been shown that PB pretreatment protects against intoxication with soman, a nerve agent that predominantly acts on the CNS (6). Finally, it appears that PB disrupts behavioral tasks that involve appropriate CNS activity [this experiment; (30,33)]. However, it cannot be excluded that behavioral performance may have been disrupted because of PB's effects on other systems intricately involved with learning and memory such as those that mediate motivation and motor activity.

In summary, the results of the present experiment show that repeated PB administration disrupts response acquisition in male and female Sprague–Dawley rats. They also indicate that such disruption is not observed after PERM administration, and that there is no significant behavioral interaction when PB and PERM are simultaneously administered. However, PB differentially affects serum PERM levels in male and female rats. Even though these sex-dependent neurochemical effects did not appear to have significant behavioral consequences under the present experimental conditions, it would be appropriate to further evaluate the differential contribution of gonadal hormones to the behavioral and neurochemical effects of PB and PERM in future experiments.

ACKNOWLEDGEMENTS

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