Pharmacology Biochemistry & Behavior, Vol. 38, pp. 549–553. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Acute Effects of Oral Pyridostigmine Bromide on Conditioned Operant Performance in Rats[1]

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Received 30 May 1990

SHIH, J.-H., W.-F LIU, S.-F LEE, J. D. LEE, C. MA AND C.-H. LIN. *Acute effects of oral pyridostigmine bromide on conditioned operant performance in rats.* PHARMACOL BIOCHEM BEHAV **38**(3) 549–553, 1991.—Pyridostigmine bromide (Pyr), the current drug of choice in the management of myasthenia gravis, has been suggested for use in Alzheimer's dementia, and as a prophylactic treatment for intoxication with organophosphate cholinesterase inhibitors. The present study was undertaken to evaluate the dose-response and time-course effects of acute oral administration of Pyr over a broad dose range (3–40 mg/kg) on the lever pressing of rats maintained under a multiple fixed-ratio (FR-20) time-out schedule of reinforcement for water reward. The drug produced a dose-dependent biphasic response depression in the overall rate of FR responding. Low doses of Pyr (≤12 mg/kg) that caused no gross signs of toxicity only moderately decreased rates of responding, primarily due to a decrease in response rates. Whereas high doses of Pyr (>24 mg/kg) which produced overt signs of peripheral cholinergic intoxication markedly suppressed overall responding, primarily due to cessation of responding. The lowest effective dose of performance disruption was 6 mg/kg, and the ED_{50} was calculated as 23.3 (17.9–28.7) mg/kg. The time-course data of performance disruption showed that low doses of Pyr (≤12 mg/kg) had an onset latency within 40–80 min and a duration of 20–80 min, whereas high doses (≥24 mg/kg) had an onset latency of 20–40 min and a duration greater than 80 min. These results suggest the recommended human therapeutic or prophylactic regimen of 30–120 mg Pyr, orally taken each 8 hours, might adversely affect behavioral performance.

Pyridostigmine Operant performance Multiple FR-TO schedule Cholinesterase inhibitor

PYRIDOSTIGMINE bromide (Pyr), a quaternary carbamate compound, is currently the drug of choice in the management of myasthenia gravis (5,16). Recently, the drug has been investigated for use in Alzheimer's dementia (1,14), and as a prophylactic treatment for intoxication with organophosphorus (OP) cholinesterase inhibitors (6, 9, 10, 13). These therapeutic or prophylactic uses are thought to rely upon Pyr's slow acting, reversible inhibition of acetylcholinesterase (AChE) and/or its direct effect on nicotinic acetylcholine receptors at the neuromuscular junctions (2, 3, 15).

In spite of its extensive study on the behavioral effects of Pyr via parenteral injections, the preclinical experiments carried out using oral administration have been limited (7). In keeping with the intended usage of this drug as a pretreatment for OP toxicity and other conditions, the oral route was employed in the present investigation. The purpose of the present study was to define the dose-response and time-course characteristics for oral Pyr on the performance of an integrated level of behavioral task in rats, in order to see if the therapeutic or prophylactic doses of Pyr (a single oral dose of 30–120 mg per person) might affect behavioral performance in man.

METHOD

Subjects

Twenty-eight experimentally naive, male Sprague-Dawley rats, initially weighing 200–250 g, were used in the experiment. The animals were individually housed in a temperature-controlled room with a 12-h light-dark cycle (light 6:00 a.m. to 6:00 p.m.). A 22-h schedule of water deprivation was used in the experiment. Food was continuously available in the home cage.

Apparatus

Eight identical operant conditioning chambers, each equipped

[1]This work was presented in part at the Fifth Annual Joint Conference of Chinese Biomedical Sciences, Taipei, Taiwan, Republic of China, April 7, 1990.

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with a response lever and water-dipper mechanism, were used. Each chamber was housed in a larger sound-attenuating shell equipped with a blower fan for ventilation. A 10-W houselight was located above each chamber's transparent ceiling. Events were scheduled by an Omron C-20 controller (Japan) and recorded by means of digital counters and two 5-channel minirecorders.

Drugs

Pyridostigmine bromide (Pyr) (mol.wt. = 261.14), 1-methyl-3-hydroxypyridinium bromide dimethylcarbamate, was synthesized by Shu-Fen Lee of the Organic Chemistry Unit of our Chemical Systems Division. Elemental analysis was satisfactory. The sample was over 99% pure as shown by high-pressure liquid chromatography (HPLC) analysis. Pyr was dissolved in distilled water and given as its salt. Pyr was administered orally by gavage at an ingestion volume of 5.0 ml per kg of body weight.

Experimental Procedure

All subjects were trained to lever press for water reward under a multiple fixed ratio time-out schedule of reinforcement (Mult FR-TO). This schedule was composed of fixed-ratio (FR) components, during which each multiple of 20 lever presses produced a drop of water (0.01 ml), and time-out (TO) components during which no reinforcement was available. The FR and TO components were alternated regularly every five minutes, signalled by the on (FR) and off (TO) of the houselights. There was a total of twelve of each component, resulting in a 120-minute session. Experimental sessions were conducted 5 days per week. The rats were maintained under these conditions for 60 daily sessions and were acclimated to oral gavage prior to the dosage regimens in order to insure a stable baseline of responding. However, two rats with the lowest response rates were dropped from the experiment.

Once responding in the mult FR-TO schedule was stabilized during each of the 12 FR components, rats were randomly allocated into seven groups (N = 3–4/group). Graded doses of Pyr (3, 6, 12, 24, 30 and 40 mg/kg) or distilled water were given in each group assigned. Throughout the experiment, Pyr and vehicle control were administered orally by gavage immediately prior to experimental sessions. The rats were normally dosed with Pyr on Thursdays; the data obtained on Wednesdays served as control data. To maintain a more normal water/food balance, an additional 10 min free access to drinking water was made available in the home cage immediately following operant sessions.

Our pilot acute oral toxicity study revealed that the LD_{50} of Pyr was 80 mg/kg. Low doses of Pyr up to 20 mg/kg (i.e., 25% LD_{50}) did not cause overt symptoms of cholinergic intoxication, such as lacrimation or chromodacryorrhea, salivation, urination, defecation, and muscle fasciculations, whereas higher doses (>20 mg/kg) did. Therefore, symptomatology was checked after each drugged session by observation.

Data Analysis

Overall response rates of the FR and TO components, and the response rates within each discrete 5-min FR period for each rat's session, were recorded daily.

The overall data, expressed as percentage of predrug control values, were subjected to a between-groups one-way analysis of variance (ANOVA) followed by a Newman-Keuls' multiple comparison test, to assess the acute effects of graded doses of Pyr.

To measure the time-course of Pyr's effects on FR responding, data for the response rates during each of the twelve, 5-min FR-20 periods was analyzed by a between-groups two-way ANOVA. Post hoc comparisons were also made using Newman-Keuls procedure.

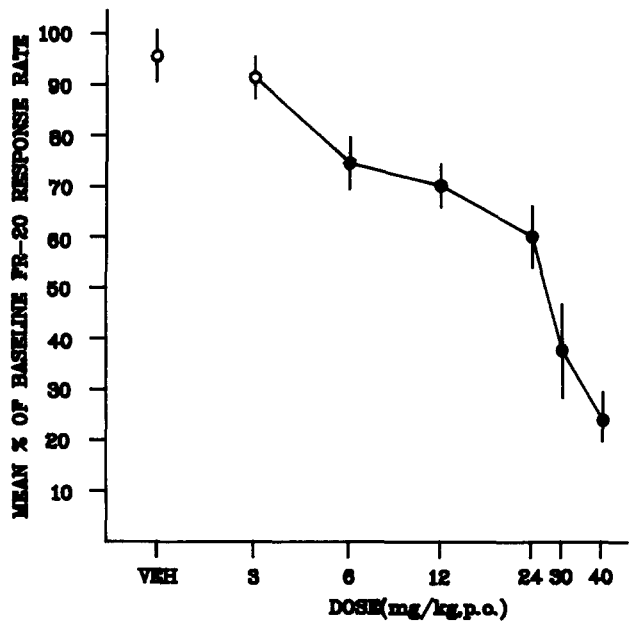

FIG. 1. Effects of oral pyridostigmine bromide on schedule-controlled responding. Each point represents the mean (± S.E.M.) percentage change from fixed-ratio preingestion baseline response rates of the 120-min session. The solid circles indicate significant differences from control values as determined by the Newman-Keuls test. N = 3–4/dose group.

ED_{50} values and 95% confidence limits were calculated from log dose-effect functions by nonlinear regression program using a two-parameter (slope, ED_{50}) sigmoidal curve-fitting program for graded data or probit analysis for quantal data.

RESULTS

The average overall control rates of responding (± S.E.M.) during successive, alternate segments of FR and TO components of the 120-min session for the 26 rats were calculated as 5376 ± 492 for FR and 22.7 ± 3.0 for TO components, respectively. In view of the very low response rate that occurred in the TO component, only data for the FR component are presented. Within this component, the response rate for all animals in the absence of drug was relatively stable during the course of the session.

The overall effect of Pyr on FR performance, evaluated over a broad dose range (3–40 mg/kg) and expressed as a percent of baseline control response rate, is shown in Fig. 1. By visual inspection of this figure, a nonlinear Pyr log-dose vs. % response depression relationship could be described ($r = -.415$, $p > 0.05$). In fact, two separate significant log-dose-% response curves for low doses (≤24 mg/kg; $r = -.767$, $p < 0.001$, $S = 37.2$) and high doses (≥24 mg/kg; $r = -.761$, $p < 0.01$, $S = 156.6$) respectively, were observed. A between-group one-way ANOVA revealed a significant effect of Pyr treatment, $F(6,19) = 17.82$, $p < 0.001$. The lowest dose of Pyr which significantly suppress this behavior was 6 mg/kg (7.5% LD_{50}) ($p < 0.05$). Multiple comparisons by Newman-Keuls test revealed that the mean percent response changes of 6, 12 and 24 mg/kg were not significantly different. High doses of Pyr (24–40 mg/kg) (30–50% LD_{50}) produced reliable reductions in the number of responses emitted from about 60% of control level for 24 mg/kg down to 25% of control response for 40

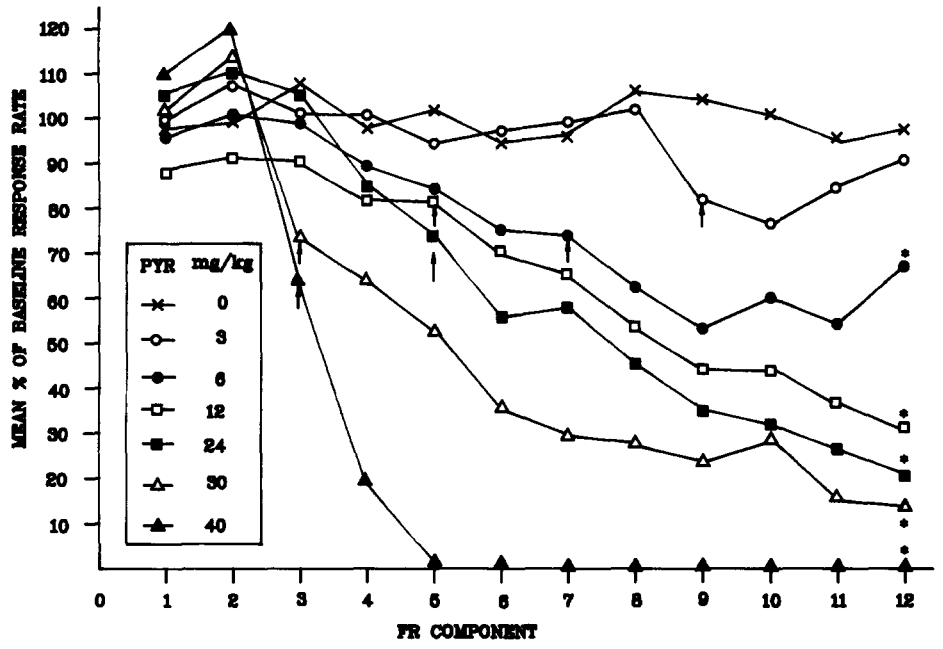

FIG. 2. Effects of oral pyridostigmine bromide on schedule-controlled responding in consecutive, alternative segments of the 5-min FR periods of the 120-min mult FR-TO experimental session. Each point represents the mean percentage change from FR preingestion baseline response rates of each respective FR component. Data of the time interval between ↑ and * indicate significant differences from their respective control values. N = 3–4/dose group.

mg/kg of Pyr. The ED_{50} (95% CL) was calculated as 23.3 (17.9–28.7) mg/kg over the broad dose range measured.

The time-course effect of Pyr on FR performance was analyzed from the 12 exposures to the 5-min FR periods in the 120-min session and is shown in Fig. 2. A between-group two-way ANOVA (dosages × time periods) revealed significant effects for dose, $F(6,19) = 21.7$, $p<0.001$, time of observation, $F(11,209) = 27.84$, $p<0.01$, and the dose × time interaction, $F(66,209) = 6.61$, $p<0.01$. Comparisons by the Newman-Keuls' procedure revealed that the mean group response rate at 3 mg/kg Pyr was not significantly ($p>0.05$) below the control level during exposure to the FR, except for the ninth and tenth 5-min periods when it was significantly depressed as compared to the correspondent vehicle control rate. At 6 mg/kg, the mean response rate was significantly ($p<0.05$) below the control level from the seventh 5-min period up to the end of the FR session. At 12 and 24 mg/kg both were significantly ($p<0.05$) lower than control from the fifth 5-min FR period up to the end of the session. At both the highest two doses (30 and 40 mg/kg), the response rate was significantly below control levels from the third period until the end of the session. Inspection of Fig. 2 reveals a small increase in responding during the first two 5-min FR periods following the three high doses of Pyr, but this is statistically insignificant with respect to the control group. The time-course data as described above and the time-course effects of the incidence of cessation of responding caused by high Pyr doses are arithmetically summarized in Table 1. These data indicate that both the magnitude and duration of the suppressive effect of Pyr on FR performance were dose related; the latency to onset of depression also differed between doses.

Inspection of the flow-chart recordings on each of the FR components (data not shown) showed that the suppressive effects of low Pyr doses (≤24 mg/kg) were primarily due to a decrease in response rates, whereas at high doses (>24 mg/kg) the disruptive effects were primarily due to long pauses in responding (i.e., cessation of responding). Observation of the symptomatology following the experimental sessions indicated that rats receiving the lower doses of Pyr (3–12 mg/kg) showed no gross signs of toxicity, whereas rats exposed to high doses (24–40 mg/kg) exhibited some overt signs of peripheral cholinergic intoxication, such as soft faeces or diarrhea, chromodacryorrhea, salivation and/or muscle fasciculations, in proportion to these doses. The proportion of rats in the group exhibiting the signs were 1/4, 2/3, 3/3 for 24, 30, and 40 mg/kg, respectively. The TD_{50} (i.e., the dose-eliciting evidence of cholinergic symptoms in 50% of the rats), analyzed by probit analysis, was calculated as 27.8 (21.7–36.3)

TABLE 1

TIME-COURSE EFFECTS OF PYRIDOSTIGMINE-INDUCED OVERALL DEPRESSION AND CESSATION OF RESPONDING UNDER A MULTIPLE FR-TO SCHEDULE OF WATER REINFORCEMENT IN RATS (N = 3–4/GROUP; TIME IN MINUTES, MEAN ± S.E.M.)

		Overall FR Depression		Pause of Responding		
Dose (mg/kg, PO)	N	Onset	Duration	No. Rats Paused	Onset	Duration
0	4	—	—	0	—	—
3	4	80	20	0	—	—
6	4	60	60	0	—	—
12	4	40	80	0	—	—
24	4	40	80	1	50.0	70.0
30	3	20	100	2	35.0 ± 5.0	85.0 ± 5.0
40	3	20	100	3	36.7 ± 3.3	83.3 ± 3.3

mg/kg. Moreover, in the rats exhibiting symptoms, these were accompanied by the presence of the cessation of FR responding, indicating that the disruption of operant performance induced by high doses of Pyr is due mainly to peripheral cholinergic poisoning. In fact, the TD_{50} obtained from the data noted above is quite comparable to that analyzed by linear regression from the graded data of FR performance disruption following high Pyr doses regimen, i.e., 26.6 (19.2–34.0) mg/kg.

DISCUSSION

The results of the present study demonstrate that acute oral administration of Pyr in rats over a broad dose range (3–40 mg/kg) produced a dose-related biphasic decrement in operant responding maintained under a mult FR-TO schedule of water reinforcement. At low doses (6 and 12 mg/kg) which did not cause any gross signs of toxicity, Pyr only moderately decreased overall response rate (i.e., below 40% of control) primarily via a general decrease in the overall rate of responding. At high doses (24–40 mg/kg) which produced overt symptoms of cholinergic intoxication, Pyr markedly suppressed the overall response rate (i.e., beyond 40%) primarily via the cessation of responding (see Table 1). The time course of Pyr's action, including latency of onset and duration of action was dose related (see Fig. 2 and Table 1).

The acute behavioral effects of the low dose levels of Pyr ($\leq$12 mg/kg, i.e., $\leq$15% LD_{50}) which produced no overt somatic signs of intoxication are in agreement with the report of Wolthuis and Vanwersch (18). They found that, intraperitoneally giving Pyr to rats, at low doses ($\leq$10% LD_{50}), interfered with several types of behavior thought to involve cortical processes, such as active avoidance, open-field behavior and the hurdle-stepping task. Based on their findings, they suggested that Pyr may have more central actions than what is commonly believed. Hence, the precise mechanism by which Pyr, as a quaternary ammonium salt that may cross the blood-brain barrier with difficulty, caused behavioral disturbance in relatively low doses is presently not known. However, the possibility exists that one or more active metabolites (e.g., 3-hydroxy-N-methyl pyridinium) (17) may have caused the central cholinergic effect (1), or the peripheral physiological effects such as gastrointestinal disturbances (5,11) may affect behavioral performance, or the direct action on the nicotinic sites of neuromuscular junction (2, 3, 15) may affect motor function.

The occurrence of cessation of responding produced by high Pyr doses (24–40 mg/kg) seemed to coincide with the presence of overt symptoms and the ED_{50}'s estimated from both the graded disruption of FR performance and the incidence data of symptoms induced were almost identical. On the basis of these findings it is tempting to suggest that the disruption of operant performance produced by high doses of Pyr may have been caused by a predominantly peripheral cholinergic action of the compound.

Based on the correlation of rat to human dosage extrapolation, in terms of amount per square meter of body surface as suggested by Freireich et al. (8), the dosage in a human would be about 7 times less than the dose in a rat on a mg/kg basis. Thus the observed behavior-deficit-free, minimal behavior-effective and sign-free doses of 3, 6 and 12 mg/kg, respectively, in rats obtained from the present study would be correspondent to a single oral doses of 30, 60 and 120 mg for a 70 kg man. These doses are within the prophylactic or therapeutic levels currently used in OP poisonings (9), myasthenia gravis (5,16) and Alzheimer's dementia (1,14). The predicted dose to cause cholinergic symptoms in man would be 240 mg. In healthy men, a single oral ingestion of 60 mg Pyr was reported to have several adverse effects such as fatigue, heavy eyelids, and gastric distress in a portion of the subjects (5). Repeated ingestion of 30 mg Pyr every 8 hours for 3 days was reported to have an effect on psychomotor performance (4), and for 4 days caused mild to moderate gastrointestinal disturbances (11). However, in elderly patients with Alzheimer's dementia, at a fixed dose of 60 mg orally, four times in 26 hours, none of the patients were reported to have any side effects such as nausea, vomiting, or diarrhea (14). These limited human data are, at least in part for healthy humans, somewhat comparable to the predicted estimates. Hence, our results imply that the recommended prophylactic or therapeutic regimen of 30–120 mg Pyr orally, taken every 8 hours, might adversely affect the behavioral performance in man, especially for the elderly patients with myasthenia gravis or Alzheimer's dementia where high therapeutic doses ($\geq$120 mg) are needed to cause beneficial effects on muscle weakness or cognitive function, respectively. Obviously, the present study provides important information about potential high-dose effects of Pyr that are of clinical importance.

A literature survey on the existing data of clinical safety evaluation of oral Pyr in therapeutic levels consistently reported that it has several subjective side effects. The most frequently reported one is gastrointestinal (GI) distress, which was not related to Pyr plasma levels (5,11), albeit that a single dose of 60 mg Pyr was reported to be the maximal dose tolerated in the GI tract in man (12). Obviously, this side effect is likely due to the increased gut motility resulting from the Pyr's anticholinesterase activity since Pyr is relatively poorly adsorbed from the GI tract (5,17). In view of this above mentioned clinical evidence, it is tempting to suggest that Pyr in therapeutic dosages may more or less adversely affect military performance, which involves highly skilled and coordinated activity, presumably as a result of GI disturbance. However, a single oral dose of 60 mg Pyr was reported to be without deleterious effect on stationary visual function (12), but a daily dosing regime did cause minimal alterations in visuo-motor coordination (4). Moreover, in keeping with our present findings via the oral route, Wolthuis and Vanwersch (18) also found behavioral effects in rats treated with IP low doses of Pyr. Accordingly, it is possible that the inhibition of AChE in the gut may interfere in a nonspecific way with many behaviors. Studies addressing this possibility are now in progress in this laboratory.

ACKNOWLEDGEMENTS

The authors wish to express their thanks to Tein-Far Chien and Ping-Tzu Liu for assisting with the experiments, to Liao-Horn Liau for typing the manuscript, and to Dr. John M. Beaton, Department of Psychiatry and Behavioral Neurobiology, at University of Alabama at Birmingham for providing valuable comments.

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