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Effect of BMY 21502 on Acquisition of Shape Discrimination and Memory Retention in Monkey

L. JAIME FITTEN, KENT M. PERRYMAN, JERRY A. HANNA AND M. K. MENON

UCLA San Fernando Valley Program in Psychiatry and Medicine
Sepulveda Veterans Administration Medical Center
Section on Geropsychiatry 116A-9, 16111 Plummer Street, Sepulveda, CA 91343

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FITTEN, L. J., K. M. PERRYMAN, J. A. HANNA AND M. K. MENON. *Effect of BMY 21502 on acquisition of shape discrimination and memory retention in monkey.* PHARMACOL BIOCHEM BEHAV **35**(3) 553–556, 1990.—BMY 21502 is a novel pyrrolidinone nootropic with demonstrated ability to reverse electroconvulsively induced amnesia in rodents. We administered BMY 21502 intramuscularly to four monkeys *(Macaca radiata)* during testing using two separate paradigms. The first test involved the acquisition of a visual shape discrimination task where each monkey learned to select the correct lighted panel. In the second task, memory retention was tested by having the monkeys select and press the correct lighted panel using a delayed matching-to-sample procedure. A dose-response relationship was established for the acquisition of shape discrimination for each monkey. Two performance-enhancing doses in the visual discrimination task were then employed to test for effects on memory retention at different delay intervals in the delayed-matching-to-sample task. Results indicate that BMY 21502, when administered over a wide dose range, enhanced acquisition of shape discrimination in three of four monkeys when combined drug scores were compared to vehicle-only scores ($p<0.02$). However, BMY 21502 produced no significant improvement in memory retention at any of seven different delay intervals when low-dose and high-dose scores for the three responding monkeys were compared to vehicle-only scores.

BMY 21502 Nootropic Acquisition Memory Monkey

THE search for effective pharmacologic treatment of age- and disease-related cognitive decline has resulted in experimentation with a broad range of investigational drugs. Among the classes of compounds receiving considerable attention have been the so-called "nootropic" agents. These are represented principally by piracetam and its analogues (e.g., amiracetam, oxiracetam, pramiracetam). The prototypic nootropic, piracetam, a substituted pyrrolidinone, is an analog of gamma aminobutyric acid (GABA) and has clear effects on brain metabolism, facilitates performance on measures of learning and retention in rodents, and protects against hypoxia-induced memory impairment in animals (4). When chronically administered to aged monkeys, modest improvement in memory performance has been reported in some subjects (1). However, controlled clinical studies in AD and other age-related cognitive disorders have been equivocal and suggest no clear pattern of cognitive improvement (2, 3, 5).

A novel pyrrolidinone derivative, BMY 21502, has recently been identified as an agent capable of reversing electroconvulsive shock-induced amnesia in rodents (8). It is currently entering phase I clinical trials as an agent for the treatment of cognitive disorders. Here we report our experience with BMY 21502 as an acquisition-enhancing drug in adult monkeys.

METHOD

Subjects

Subjects for this study were four healthy female colony-born *Macaca radiata* (bonnet) monkeys obtained from the Davis Regional Primate Center and TB tested every six months. The monkeys' ages were 7, 13, 18 and 24 years (species lifespan in captivity ca. 25–30 years) and their weights varied from 4.0 to 5.2 kilograms. All monkeys were individually caged and fed a diet of moistened old-world monkey chow supplemented by fruit. Water intake prior to testing was restricted to 100 ml/day on weekdays (test days). Immediately after testing and on weekends water was supplied ad lib.

Test Procedures

Discrimination learning. A shape discrimination task was used for the acquisition phase of this study. Two side-by-side, computer-controlled stimulus-response (S-R) panels were employed for the visual discrimination tests. For one week prior to training, each subject was adapted to the test apparatus and shaped to panel press for liquid reinforcement. All subjects had previous behavioral test

experience in the same apparatus. Training consisted of exposure to a pair of pattern stimuli with consistent reinforcement to one pattern which alternated from side to side in random sequences. After three weeks of training, a shape discrimination trial began with each rear projection S-R panel being illuminated with a different geometric shape (e.g., triangles, squares, circles, lines). The same two geometric shapes were presented for each block of 25 trials until the monkey reached learning criterion, whereupon a new set of stimuli were selected. One of the two shapes was consistently reinforced throughout these trials. Shapes alternated in a pseudorandom fashion between the two panels. The monkey was seated in a Forrenger chair at arm's length from the S-R panels. A trial began with the onset of a 400 Hz tone lasting 5 seconds. Thereafter, the panels were illuminated with the shapes. Depressing the correct panel within 22 seconds of the onset of the stimulus produced immediate reinforcement (1 ml of Hi-C Fruit Drink) via a chair mounted delivery nozzle placed adjacent to the monkey's mouth. Delivery of the reinforcement fluid was under computer control utilizing a Davis syringe pump. Responses with a latency greater than 22 seconds were treated as an omission. Five seconds following an error or an omission, the computer began another trial. Intertrial intervals between correct responses were 6 seconds. Each daily session of shape discrimination consisted of four blocks of 25 trials, each using the number of correct trials per block as the index of learning. Learning criterion was defined as a minimum of 88% correct discriminations on three successive blocks.

Memory retention. A delayed matching-to-sample paradigm was used to test recent memory. For this, a third, stimulus-only, rear-projected panel, positioned between the two S-R panels, was used to display the sample stimuli, which consisted of two pseudorandomly alternating shapes. The two adjacent S-R panels served to display the target stimulus and offer the monkey a response choice. The correct target stimulus alternated between these two S-R panels in a pseudorandom fashion. Each trial began with the onset of a 400 Hz tone (5 seconds). At the end of the warning tone, the center stimulus panel was illuminated for three seconds with the sample shape. This was followed by a delay period which varied from 2 to 24 seconds, depending on the delay interval used in that trial. If the monkey pressed the unlit S-R panel during the delay period no reinforcement was provided. Following the delay interval, both adjacent S-R panels were illuminated with geometric shapes, one of which was the same as the sample figure. The monkey was allowed 10 seconds to make a response. Correct responses (matching to the sample panel) were rewarded immediately. A 5-second intertrial interval followed correct responses. Incorrect choices and omissions resulted in no reinforcement with a 5-second time-out period. Retention was indexed as the number of correct matches per 100 daily trials. A longer delay was introduced each day and the sample stimuli changed. The delays used in this study were 2, 4, 8, 12, 16, 20, and 24 seconds.

Drug Administration

Discrimination learning. BMY 21502 was obtained from Bristol-Myers Company, Evansville, IN (Fig. 1). The drug was dissolved in a vehicle of bacteriostatic water and dimethyl foramide in a ratio of 9 to 1. Injections (IM) of drug or vehicle were given 1 hour prior to testing except for doses below 2.1 mg/kg/day which were administered ½ hour prior to testing.

Before administering drug, predrug learning performance was determined after the adaptation and shaping process. Thereafter, a dose-response relationship for BMY 21502 was established. All monkeys were begun on 0.02 mg/kg/day of drug and tested daily on that dose until they reached learning criterion on 3 successive

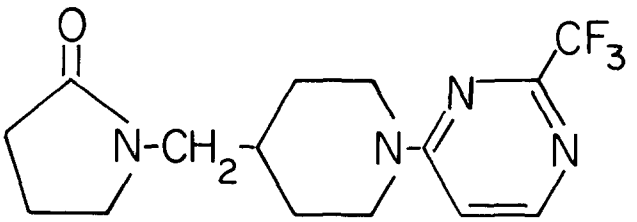

FIG. 1. BMY 21502 [1-(1-(2-(trifluoromethyl)-4-pyrimidinyl)-4-piperidinyl)methyl)-2-pyrrolidinone].

days. As soon as criterion was met, the dose was doubled until 5.12 mg/kg was reached. Thereafter, the dose was increased by increments of 2.5 mg/kg. This procedure continued until performance deteriorated, as evidenced by an increasing number of trials needed to reach criterion. At this point (onset of behavioral toxicity), drug administration was stopped. The testing of each dose was followed by two to three drug-free days. Five separate vehicle-only periods, each preceded by a two-day drug-free interval, were included during the drug trials. Effective and behaviorally toxic doses were identified on the basis of the number of trials needed to reach criterion. Effective doses determined here were later used in the memory retention, delayed matching-to-sample paradigm.

Memory retention. For each monkey, a low and a high dose was chosen from within the previously determined effective learning dose range. A low-dose/high-dose sequence was used with each set of stimuli. For each dose, the full range of delays was tested by a modified, ascending geometric progression paradigm. Only one delay interval and set of stimuli was used per day. These stimuli have been used successfully in previous studies employing young and aged macaque monkeys. The number of correct choices for each delay was recorded and constituted the retention score. Drug retention scores were compared to baseline and vehicle-only scores.

Statistics

The Student's *t*-test was used to evaluate drug effects on performance during the acquisition phase. A factorial design was employed to analyze the results of the memory retention phase, which contained multiple doses and multiple delays.

RESULTS

Determination of Effective Doses

An effective dose range for each subject was identified based on best performances, i.e., fewest trials to criterion. Effective dose ranges were broad and varied from subject to subject as did the doses which indicated behavioral toxicity. Monkey No. 16757 (18 years old) did not show a U-shaped dose-response curve as did the other three subjects (Fig. 2).

Shape Discrimination Task (Acquisition)

During the nondrug conditions, the youngest monkey (S2) demonstrated the fastest rate of acquisition. The difference between this young monkey and the three responders diminished during the BMY periods with the latter improving from their nondrug performances. Figure 3 compares performance (mean trials to criterion) of the 4 monkeys during the BMY 21502 and vehicle-only conditions. Performance on drug is the average of all

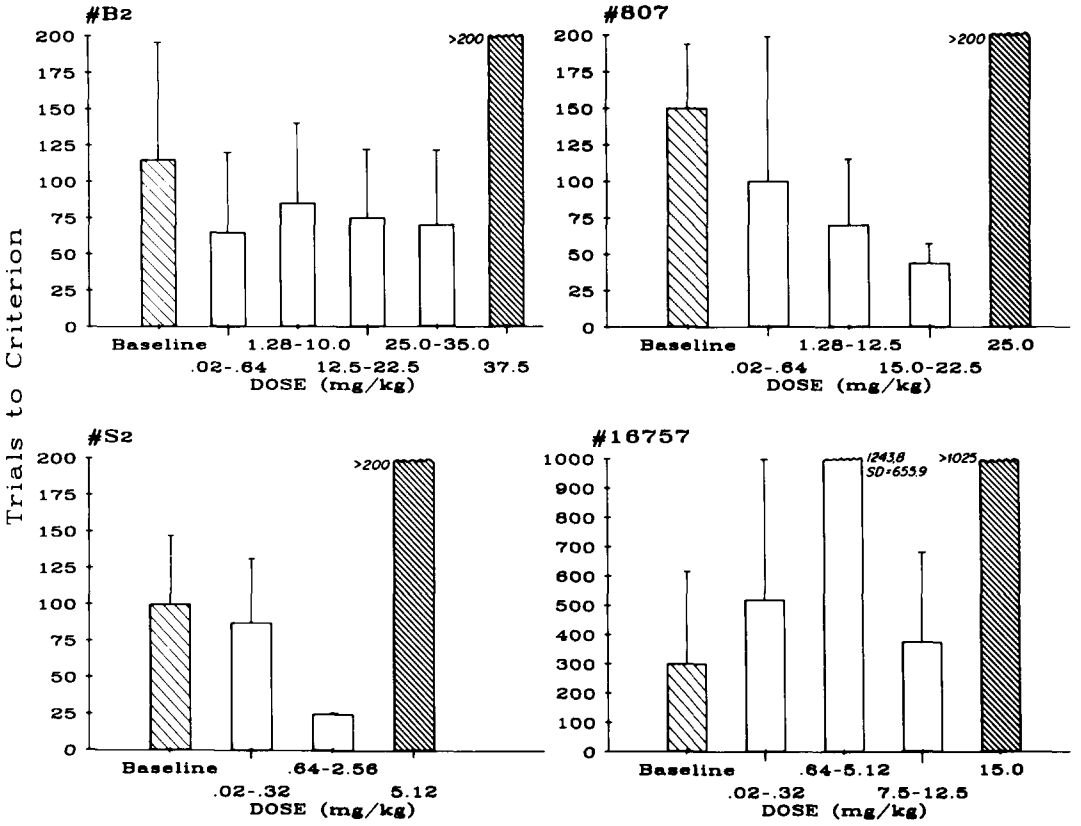

FIG. 2. Dose-response relationships. Three of four monkeys demonstrate improved acquisition (fewer trials to criterion) of a visual discrimination task under increasing doses of BMY 21502 until an individual behaviorally toxic dose is reached (shaded bar at right). The fourth monkey (No. 16757) performs less well initially (baseline) and appears behaviorally impaired by the drug. Vertical lines represent the standard deviation.

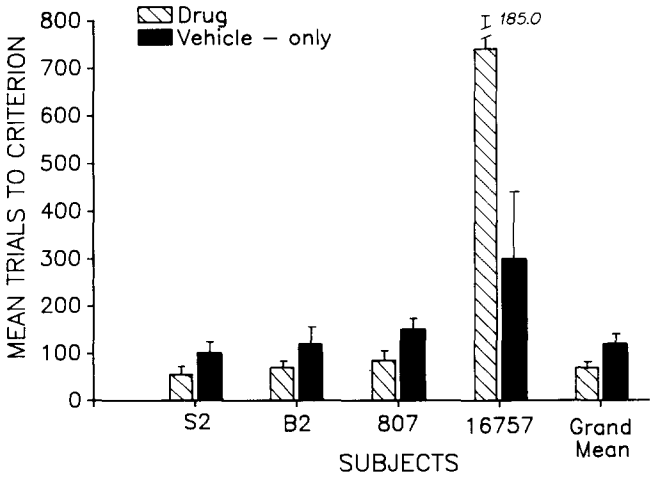

FIG. 3. Acquisition of shape discrimination. Three of the four monkeys learned to make the shape discrimination requiring fewer trials while on drug (BMY 21502). The grand mean of the three responding monkeys demonstrates a significant difference between the drug and vehicle-only conditions ($p<0.02$). Monkey No. 16757 was unable to acquire the discrimination task. Vertical lines represent the standard error of the mean.

doses below behaviorally toxic levels (effective dose range 0.02 mg/kg/day–35.0 mg/kg/day IM). Three of four monkeys required fewer trials to reach criterion while on drug when compared to the vehicle-only condition. No clear learning pattern emerged during successive vehicle-only trials.

The combined vehicle-only scores and the combined drug scores for these 3 subjects (grand means) were compared using Student's *t*-test. A significant difference was found at the $p<0.02$ level. Under the animal's best dose, monkeys' Nos. B2, 807 and S2 percent improvement (fewer trials to criterion) over baseline was 43%, 67% and 75% respectively. The youngest monkey thus demonstrated the greatest magnitude of improvement. The fourth monkey was not able to learn the task while on drug, and its performance actually seemed impaired when compared to the nondrug condition.

Delayed Matching-to-Sample (Memory Retention)

For the vehicle-only trials, the youngest monkey consistently demonstrated the highest percent retention scores of the group. For the overall memory-retention paradigm, statistical analysis was based on a two-factorial design with three levels of drug and seven levels of delay. No significant main or interaction effects were found.

DISCUSSION

These results indicate that BMY 21502, when given over a

relatively wide dose range, effectively enhanced acquisition of shape discrimination in three of four monkeys. The determination of effective doses for each monkey was necessary since considerable variability was found from one monkey to the next. BMY 21502 was well tolerated even at higher doses. No overt adverse effects were observed. Only a rapid deterioration of performance indicated toxicity at the upper range of doses. A U-shaped dose-response relationship was found for each of the three responding monkeys. The fourth monkey failed to respond consistently at all doses tested and may have been impaired by the drug. Age could have been a factor in this diminished performance. While not the oldest, this monkey may be representative of an older population of subjects. Greater intersubject variability in drug sensitivity and/or behavioral performance would be expected with advancing age. Thus, for a given group of older monkeys, the most drug sensitive or the poorest performer may not invariably be the oldest. Due to the small sample size in this study, age-related variability cannot be adequately assessed.

No significant memory retention improvement was obtained with the doses selected from the acquisition paradigm. Doses effective in enhancing learning may be ineffective in improving short-term memory retention. Marriott *et al.* (6), using the nootropic CI-933 in aged monkeys, also found improved cognitive performance, but not under conditions of delayed recall. These authors concluded that CI-933 has no direct effect upon recent memory, but does affect attentional, motivational or learning components of the task. Other studies have reported on the effects of a variety of nootropics administered to monkey (1,7). These studies demonstrated some improvement in memory performance. However, not all subjects showed the same qualitative response. Some clearly improved while others showed no change. In a few cases, mild impairments were reported.

Based on our experience, BMY 21502 appears to share several characteristics with other agents of this class. Improvement in the performance of nonhuman primates is modest and does not occur in all subjects. Learning rather than short-term memory, is enhanced, and individualized dosing is required to elicit improved test performance. Memory retention performance has been the focus of interest in many investigations relating to cognitive decline. However, facilitation of learning, if sufficient, could have desirable clinical outcomes. This would be true of patients with cognitive deterioration such as that found in AD, provided not all storage function has been lost.

Finally, we cannot suggest from our data, nor, from other currently available findings, the mechanism by which acquisition of shape discrimination in monkeys is enhanced. It is possible that BMY 21502 exerts its effects by augmenting general arousal, attention or motor performance, thus influencing indirectly, though effectively, the learning process.

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