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BRIEF COMMUNICATION

The Benzodiazepine Receptor Inverse Agonist RO 15-3505 Reverses Recent Memory Deficits in Aged Mice

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FORSTER, M. J., P. L. PRATHER, S. R. PATEL AND H. LAL. *The benzodiazepine receptor inverse agonist RO 15-3505 reverses recent memory deficits in aged mice.* PHARMACOL BIOCHEM BEHAV 51(2/3) 557-560, 1995.—The benzodiazepine receptor partial inverse agonist RO 15-3505 was tested for its ability to improve impaired recent memory of aged mice. All mice successfully acquired a learning set for accurate identification of the correct arm of a T-maze and could perform with nearly 100% accuracy after 1-min delays. However, performance of the aged mice approached chance levels after 2-h delays. When injected just before testing on a series of 2-h retention tests, RO 15-3505 (from 2.5-10.0 mg/kg) resulted in a marked improvement of response accuracy. These results confirm the role of benzodiazepine receptor mechanisms in the modulation of memory processes, and suggest that the memory-facilitating effects RO 15-3505 or similar benzodiazepine receptor ligands may be generalized to aged rodents with impaired memory function.

Benzodiazepine inverse agonist Benzodiazepine antagonist Alzheimer's disease C57BL/6NNia mice
Aging, memory decline Working memory RO 15-3505 Flumazenil

THERE IS considerable experimental evidence indicating that benzodiazepine receptor mechanisms participate in the modulation of learning and memory processes. Whereas diazepam-like benzodiazepine receptor agonists interfere with memory processes, benzodiazepine inverse agonist and antagonist compounds have been reported to facilitate memory performance under a variety of conditions [for reviews, see (4,6,10,22,23)]. For example, the benzodiazepine antagonists flumazenil (15) and CGS-8216 (12), as well as the partial inverse agonist RO 15-4513 (20), enhance both acquisition and retention of a discriminated escape response in young mice. In addition, amnesia induced by the anticholinergic scopolamine can be reversed by flumazenil (15), ZK 93 426 (22), and RO 15-4513 (20).

It has been suggested that the cognition-enhancing effects of the benzodiazepine receptor ligands involve indirect modulation of cholinergic neurotransmission (22,23) and, therefore, the benzodiazepine inverse agonists or antagonists may prove especially useful in the treatment of Alzheimer's disease (AD) or age-associated memory impairment. Although the compounds ZK 93 426 and flumazenil have been tested in a variety of experimental models of age-related memory dysfunction (8,13,14,22), few studies have specifically addressed the ability of these or other benzodiazepine receptor antagonist-inverse agonist compounds to reverse spontaneous impairments in recent memory exhibited by normally aged rodents. In the current study, we examined the ability of the benzodiazepine receptor partial inverse agonist RO 15-3505 (17) to improve recent memory capacity in memory-impaired, C57BL/6NNia mice of highly advanced age. A delayed two-choice discrimination paradigm recently described by us (5) was used to assess

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working memory impairment and responsiveness to drug therapy using a within-subjects experimental design.

METHOD

Animals

The nine C57BL/6NNia mice used in these studies were 26 (n = 3) or 31(n = 6) mo of age at the completion of the experiments. This number represented the survivors of an initial group of 25 mice used in a previous study (5). Following their receipt from the supplier (National Institute on Aging), the mice were housed two to four per cage in 30.4 × 18 × 12.8-cm clear polycarbonate cages with food and water available ad lib. The colony was maintained at 23 ± 1°C, on a normal light–dark cycle beginning at 0700 h.

Apparatus

The apparatus for recent memory testing was an acrylic T-maze described previously (5), with compartments in the stem and goal arms, each demarcated by a removable door. The width of the stem and goal arms was 6.4 cm and the clear ceiling was 5 cm high. The stem was 20 cm long (from base to choice point) and included a start-box demarcated by a manual guillotine-type, acrylic door 10.3 cm from the stem base. Each arm of the maze could be closed by an opaque, sliding door moving flush to each outside wall of the stem. The distance from the outside wall of the stem to the end of each arm was 14.5 cm. The maze rested on a grid floor composed of 3 mm diam. stainless-steel rods spaced 7 mm center to center and wired for 0.5-mA scrambled shock from a BRS-LVE (Beltsville, MD) constant power shock source (Model SGS-003).

Procedure

In an initial series of daily training sessions, the mice learned to use information presented on a single trial (the first trial of each session) to successfully avoid shock from the grid floor on all subsequent trials of that session. Each session consisted of an information trial followed by discriminated avoidance training to a performance criterion. The discriminated avoidance training consisted of discrete trials in which shock to the grid floor was initiated 5 s following opening of the start door if the mouse had not entered the correct goal arm. Shock was initiated immediately following entry into the incorrect arm. After initiation, the shock continued (up to a maximum of 60 s) until the correct arm was entered, after which the mouse was removed and placed in a holding cage for a 1-min intertrial interval. On the information trial, the same contingencies were in effect except that the arm first entered was always incorrect, and avoidance of shock on the next trial (the test trial) required a reversal from the previous arm choice. The reversal added a simple working memory requirement for successful avoidance on the test trial.

The discrimination and avoidance components of the task were scored separately. A stem avoidance was recorded if the mouse entered either one of the goal arms within 5 s of the start door opening. A correct turn was scored when the first arm selected on a given trial was correct, regardless of the response latency. Avoidance training continued on each day until the mouse had made a correct avoidance (i.e, a correct turn as well as a stem avoidance response) on five consecutive trials. The training phase was discontinued when a mouse had made correct avoidances on the test trial and all subsequent trials in three consecutive sessions.

Prior to drug testing, the mice used in the current studies had been tested and compared with younger mice for their ability to enter the correct goal following a series of delays interposed between the information and test trials [see (5)]. This study indicated that time-dependent memory decay (from 0.25–2 h) was more rapid in the current group of mice, when compared younger mice aged 7–9 mo (5). The current report describes the effect of RO 15-3505 (Hoffman La Roche, Nutley, NJ) on performance of this group of 24–29-mo-old mice at 2-h delays, when injected intraperitoneally (IP) (0, 2.5, 5.0, or 10.0 mg/kg) 15 min before the information trial. The 2-h delay was selected because the difference in turn accuracy between young and old mice was maximal using that parameter.

The doses were presented in separate sessions in which the 2-h delay interval was presented, beginning with a vehicle session and including at least one intervening saline session between each drug presentation. Doses were presented in an incremental fashion to identify potentially disruptive or toxic effects of higher doses, and to equate animals for history of any such effects on subsequent tests. The intervening sessions involved a short (1-min) delay and were included to provide a washout period and to test baseline performance. This arrangement resulted in a minimum of 48-h elapsing between sessions in which drug was present. If the mouse failed to exhibit a correct response on the test trial of an intervening session, then additional saline sessions were conducted until the mouse had criterion performance on two consecutive sessions, before continuation of the 2-h delay drug testing sessions. Testing continued until all mice had received four cycles of drug presentation. Memory performance was assessed for the four test trials at each dose, in terms of discrimination accuracy (correct turns), and as response speed, the reciprocal of the latency (corrected by + 0.5 s) to reach the correct goal. Response speed on the last avoidance of each drug session was included as a measure of error-independent avoidance performance. Data were analyzed using analyses of variance (ANOVA) and planned individual comparisons between RO 15-3505 dose sessions and vehicle sessions.

RESULTS

The mice completed the four cycles of drug presentation over the course of 2 mo, with an average of 67.2 h elapsing between each dose presentation. An ANOVA failed to indicate significant differences in the presentation interval as a function of dose.

As suggested in Fig. 1, group data for discrimination performance of vehicle-treated mice tested at the 2-h delay was near chance level, whereas nearly 100% accuracy was evident on vehicle sessions with a 1-min delay conducted on the following day (hatched bar). The effect of delay was significant for both the number of correct turns [$F(1, 8) = 9.1$, $p = 0.017$] and response speed measures [$F(1, 8) = 44.4$, $p < 0.001$]. RO 15-3505 treatments resulted in a dose-dependent increase in response accuracy, as reflected in both the correct turn and response speed measures. The number of correct turns was significantly higher following all doses of RO 15-3505 when compared with vehicle sessions [$F(1, 24) = 9.2$, 11.7, and 20.7 for 2.5, 5.0, and 10 mg/kg, respectively; p from 0.006 to < 0.001]. Test trial response speed was also increased following 5.0 [$F(1, 24) = 7.3$, $p = 0.012$] and 10.0 mg/kg [$F(1, 24) = 7.7$, $p = 0.011$], when compared with response speed on vehicle sessions.

Only three of the mice performed at greater than chance

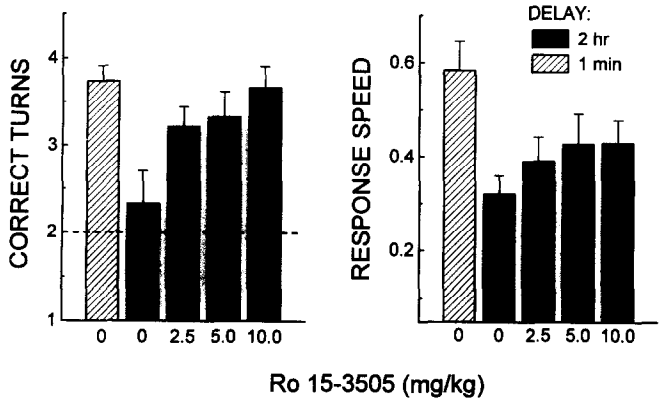

FIG. 1. Scores on retention test trials for 26--31-mo-old mice injected IP with the vehicle or RO 15-3505, 15 min before each information trial. (Left) Mean number of correct turns ± SE; the dotted line represents chance level performance. (Right) Mean response speed 1/(latency + 0.5 s) ± SE. The vehicle was injected 15 min before all sessions involving 1-min retention tests (hatched bars).

numbers of correct turns during vehicle tests (two aged 26 and one aged 31 mo). Therefore, the group effects noted earlier were largely attributable to the response of the six memory-impaired animals (the unimpaired subjects had higher levels of performance and showed little change in response to drug treatments). Each of the six memory-impaired mice made the correct turn with 100% accuracy following at least one of the doses of RO 15-3505, yet all performed at chance level following vehicle.

Analysis of error-independent avoidance response speed on the last trial of each drug session suggested that this parameter failed to vary as a function of delay. RO 15-3505 treatments were without effect on this parameter following all but the highest dose (10 mg/kg), for which a decrease in avoidance speed was detected [$F(1, 24) = 13.3, p = 0.001$].

DISCUSSION

Previous investigations provided a clear indication that in mature, normal subjects, learning and memory processes may be impaired by diazepam-like benzodiazepine receptor agonists (6,9,16,25) and improved by antagonists or inverse agonists (9,12,15,20–23,26). Other recent investigations suggested that under conditions involving deficits or abnormalities of brain function, drugs of the agonist or inverse agonist class are also beneficial. The benzodiazepine antagonist flumazenil was effective in reversing learning and memory deficits associated with autoimmunity (14) and deficits produced by the anticholinergic scopolamine (15). Moreover, both RO 15-3505 and the benzodiazepine receptor antagonist ZK 93 426 were capable of reversing learning or retention deficits induced by lesions of the basal forebrain (7,22). It appears that the beneficial effects of benzodiazepine receptor ligands can also be generalized to the aged nervous system, based on the present finding that RO 15-3505 improved working memory performance of memory-impaired aged mice.

Even though a statistically significant effect of drug was evident for the group data, it should be recognized that the individual aged mice differed in their baseline levels of recent memory performance. Within the impaired group of mice, the effects of RO 15-3505 were obtained within a wide dose range, and there was no indication that tolerance developed to the beneficial effects over the testing period. A performance ceiling prevented observation of equivalent improvement by unimpaired mice following RO 15-3505, although effects might have been observed had these mice been tested under a more difficult delay parameter. Nevertheless, it is worth noting that a relatively high dose of RO 15-3505, which dramatically improved performance of impaired mice, had no adverse effect on performance of the three aged subjects with apparently unimpaired recent memory function.

Because the current studies employed a repeated measures design, it is possible that multiple exposure to RO 15-3505 treatment somehow contributed to the observed improvement in memory performance. A significant accumulation of the drug in the impaired mice seems unlikely, given that this group exhibited impaired performance during those saline sessions occurring after each 10-mg/kg dose of the previous cycle, a point at which maximal drug accumulation would have been expected. However, the current studies do not rule out pharmacodynamic changes or behavioral adjustments as a consequence of repeated drug exposure.

Whereas RO 15-3505 has been characterized as a weak partial inverse agonist compared with other compounds of this class (19), the possibility that this compound could improve memory performance via arousal-enhancing or anxiogenic effects cannot be ruled out by the current studies. A locomotor stimulant effect of RO 15-3505 has been demonstrated in habituated mice (11), indicating that enhanced arousal or vigilance may be a consequence of RO 15-3505 treatment. If increased arousal were present in the RO 15-3505-treated mice in the present investigation, this effect was not expressed as an increase in error-independent avoidance speed. However, there is no evidence that could rule out task-specific increases in vigilance and arousal as the mechanism responsible for improved recent memory performance.

An anxiogenic or fear-enhancing effect of RO 15-3505 could also account for the current findings, although the general pattern of learning and memory performance-enhancing effects across antagonist and inverse agonist benzodiazepine receptor ligands is inconsistent with such an interpretation (20,21). In previous experiments, we demonstrated that learning and memory performance enhancement in mature mice was evident following treatment with the antagonist benzodiazepine receptor ligands flumazenil and CGS-8216, at doses unlikely to result in anxiogenic or convulsant effects. A recently completed experiment (Forster and Lal, in preparation) has confirmed that CGS 8216 is equally effective in improving recent memory performance in aged C57BL/6 mice, when compared with RO 15-3505.

The current studies did not investigate the mechanisms for the beneficial effects of RO 15-3505, although the present findings are quite consistent with the possibility that endogenous diazepam-like endocoids exhibit a tonic inhibitory influence on learning and memory processes (10,15). The existence of such endogenous agonists would account for the nearly equal efficacy of inverse agonists and antagonists in producing improvement in learning and memory performance in aversive paradigms (20).

Previous studies have suggested that the cognition-enhancing effects of benzodiazepine inverse agonists in young rodents may be related to disinhibition of cholinergic activity, produced via benzodiazepine receptor modulation of GABA neurotransmission (18,22,23). Given that memory impairment

of aged rodents has been attributed to loss of presynaptic function of neurons of the basal forebrain cholinergic system [cf. (2,3,24)], the enhancement of cholinergic neurotransmission would certainly be one likely explanation for the improved performance of aged, RO 15-3505-treated mice. A recent finding suggesting that benzodiazepine receptor inverse agonists may also facilitate release of cortical acetylcholine in aged rodents (1) adds further support to this interpretation.

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