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BRIEF COMMUNICATION

Flumazenil Improves Active Avoidance Performance in Aging NZB/BlNJ and C57BL/6NNia Mice

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LAL, H. AND M. J. FORSTER. *Flumazenil improves active avoidance performance in aging NZB/BlNJ and C57BL/6NNia mice.* PHARMACOL BIOCHEM BEHAV **35**(3) 747–750, 1990.—C57BL/6NNia and autoimmune NZB/BlNJ mice aged 12–14 months were tested for acquisition and retention of an active avoidance response following vehicle or flumazenil (40 mg/kg), a benzodiazepine antagonist. Acquisition and retention performance was improved in flumazenil-treated mice when compared with vehicle-treated mice, although the degree of improvement varied with the level of performance in vehicle-treated mice of each strain. The NZB/BlNJ mice, which generally performed more poorly than the C57BL/6NNia mice, showed the greater improvements following flumazenil. These results suggest that antagonism of benzodiazepine receptors leads to improved learning and/or memory performance in mice with spontaneous age-associated deficits.

Autoimmunity Aging Flumazenil Benzodiazepine receptor antagonist Memory Amnesia
Cognitive decline Avoidance learning NZB/BlNJ mice C57BL/6NNia mice

RECENTLY, we reported that learning and memory for a discriminated escape task was enhanced in young ICR mice following pretreatment with either flumazenil (Ro 15-1788), an imidazobenzodiazepine benzodiazepine receptor antagonist or CGS 8216, a pyrazoloquinoline-type antagonist (11,16). Because synthetic diazepam-like benzodiazepines impair learning and memory (10, 17, 29, 30), one explanation for these findings is that an endogenous diazepam-like ligand exerts a modulatory influence on learning or memory processes via benzodiazepine receptors. Alternatively, inverse agonist-like properties of these drugs could be responsible for enhancement of learning or memory performance. The prospect of modulation of memory processes via benzodiazepine receptors has led to the suggestion that benzodiazepine antagonists or inverse agonists might be useful in the therapy of age-related dementia, particularly Alzheimer's disease (11, 16, 25). As an initial step toward assessment of potential gerontological applications for benzodiazepine antagonists, the current study was undertaken to determine the potential for flumazenil to improve learning or memory in mice with age-related deficits.

Two mouse strains, C57BL/6NNia and NZB/BlNJ, were selected for study based upon their heterochronic development of learning/memory deficits during aging (4, 14, 15). For example, declines in avoidance learning of C57BL/6NNia mice are first evident by 12 months of age, whereas those of NZB/BlNJ mice begin as early as 3 months of age (3). C57BL/6 mice have been studied previously as models of age-related/memory decline [cf. (2,8)], whereas NZB/BlNJ mice are genetically autoimmune-prone (28) and, at maturity, show a number of behavioral and immunological abnormalities similar to chronologically older mice (4, 14, 15, 26, 27). The NZB/BlNJ mice also exhibit abnormal behavioral responses to several classes of drugs affecting the cholinergic system (22–24), and show an accelerated, age-related increase in sensitivity to the benzodiazepine diazepam when compared with C57BL/6 mice (6). If antagonism of benzodiazepine receptors can facilitate those brain processes involved with age-associated learning/memory decline, then it was expected that flumazenil pretreatment would reverse age-related active avoidance impairments in 12–14-month-old NZB/BlNJ and C57BL/6NNia mice.

METHOD

Animals

Twenty NZB/BlNJ mice (The Jackson Laboratory, Bar Harbor, ME) and 27 C57BL/6NNia mice (National Institute on Aging) were housed in the college vivarium from three months of age until testing at ages ranging from 12 to 14 months. While in the

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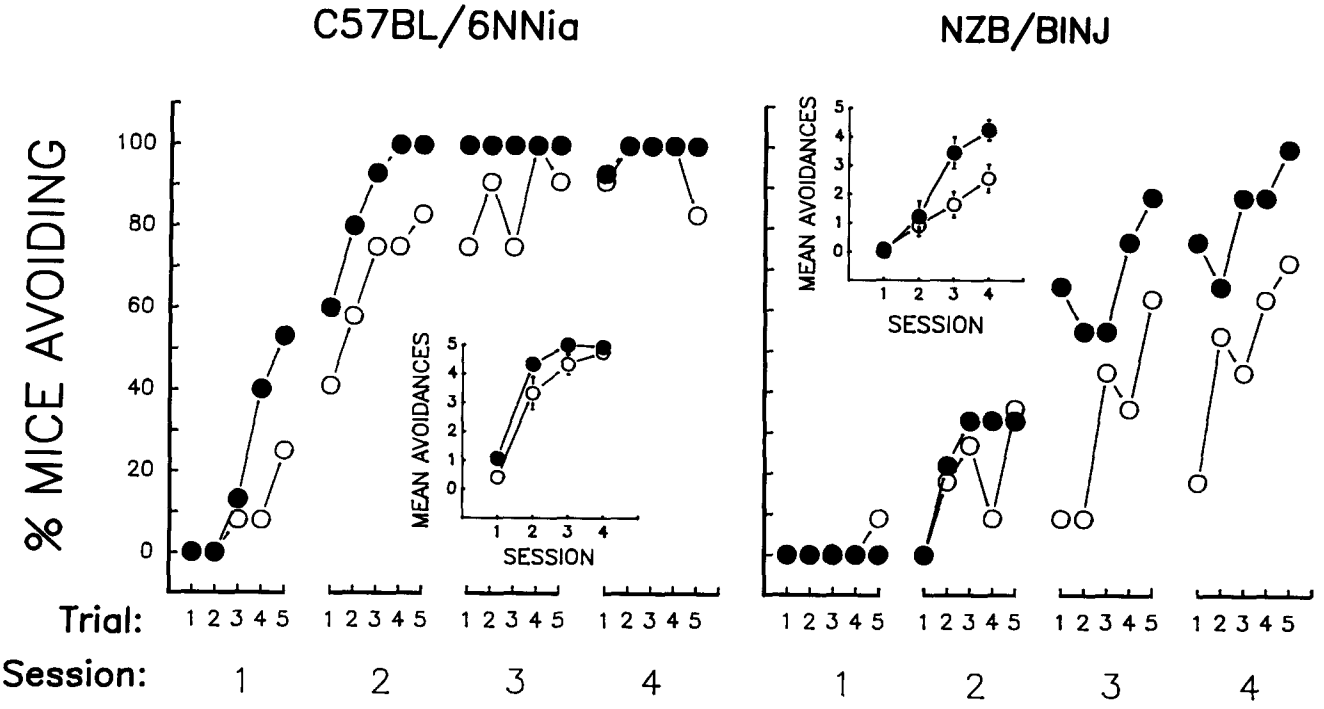

FIG. 1. Percentages of C57BL/6NNia (left) and NZB/BlNJ mice (right) exhibiting avoidance responses as a function of vehicle (○—○) or 40 mg/kg flumazenil (●—●) treatments, testing session, and individual trials. Each figure inset shows the mean number of avoidances (±SEM) within each testing session for each drug group.

vivarium the mice were housed in clear polycarbonate (28 × 19 × 13 cm) cages (3–5 per cage) with access to food and water ad lib. The colony room was maintained at 22 ± 1°C, on a normal light-dark cycle beginning at 0800 hr. All tests were conducted between 1000 and 1500 hr.

Apparatus

The apparatus (3) consisted of a 29.3 × 13.0 × 15.8 cm (i.d.) clear acrylic chamber with an open bottom. The top of the chamber was hinged 9.0 cm from the right side and there was a platform on the lower left wall of the apparatus, extending 8.5 cm from the left wall and 5.0 cm above the floor. The wall and platform were removable as a unit. The chamber rested on a grid floor consisting of 3.0-mm diameter stainless steel bars spaced 7.0 mm center to center and extending perpendicular to the length of the chamber. The bars were wired for scrambled shock from a BRS/LVE shock source (Model SGS-003). A tone generator (Mallory Sonalert Model 5C628) was mounted in the top of the apparatus.

Procedure

Flumazenil (a gift from Hoffmann-La Roche) was suspended in a solution of water (7 parts) and 3% carboxymethylcellulose (3 parts) just prior to injections. Because availability of the aging NZB/BlNJ mice was limited, one dose (40 mg/kg) was selected for study. This dose had been maximally effective in previous investigations of young ICR mice (16). Separate groups of the C57BL/6NNia and NZB/BlNJ mice received intraperitoneal injections of flumazenil (40 mg/kg) or the vehicle, 10 min prior to daily testing under a one-way active avoidance paradigm, modified from a previous investigation (3). Each mouse was given a total of 4 training sessions at 24-hr intervals, each session consisting of 5 active avoidance trials. On each training trial a 2.8 kHz, 90-dB tone was sounded just following placement of the mouse in the avoidance chamber. An avoidance response was recorded if the mouse reached the platform within 10 sec following the onset of the tone. If an avoidance response did not occur within the 10 sec, a 1.8 mA scrambled footshock was initiated which continued until the mouse reached the safe platform (or it was removed after a maximum latency of 60 sec). Each trial ended with the mouse being transferred from the avoidance chamber to the holding cage for a 60-sec intertrial interval.

In contrast to previous investigations (4), the current avoidance paradigm included both acquisition and retention components. Acquisition was evaluated in terms of the number of avoidance responses and the latency to reach the safe platform within each daily session. Retention was defined as the degree to which performance levels achieved during a given session were preserved on the initial trials of the next training session.

RESULTS

The effect of daily flumazenil injections upon active avoidance performance of the C57BL/6NNia and NZB/BlNJ mice is depicted in Fig. 1. The main figures show the percent mice avoiding as a function of trials and sessions; the insets show the mean numbers of avoidances as a function of daily sessions. Overall, the C57BL/6NNia mice showed faster acquisition and superior retention of the avoidance response when compared with the NZB/BlNJ mice. A particularly striking difference between the vehicle-treated groups of each strain was the lack of 24-hr retention of avoidance responding at the beginning of session 3 and 4 for the vehicle-treated NZB/BlNJ mice. In the vehicle-treated C57BL/6NNia mice, performance levels at the beginning of sessions 2, 3,

and 4 were unchanged or improved relative to the last trial of the previous day, suggesting good 24-hr retention. Flumazenil pretreatment tended to facilitate both within- and between-session performance in both strains, although the effect was greatest for the NZB/BlNJ mice during sessions 3 and 4.

To verify the effectiveness of flumazenil treatments, a three-way analysis of variance was conducted on the total number of avoidances within each session (see Fig. 1, insets), with Strain, Drug, and Session as the factors. A significant Strain × Drug × Session interaction, $F(3,129)=4.1$, $p<0.01$, resulted primarily from the large differences between flumazenil- and vehicle-treated NZB/BlNJ mice during sessions 3 and 4. Individual comparisons (32) between drug and vehicle groups within this interaction verified that those differences were significant, $Fs(1,43)>11.3$, $ps<0.005$. In addition, flumazenil-treated C57BL/6NNia mice made more avoidances than their vehicle-treated controls during the first avoidance session, $F(1,43)=4.5$, $p<0.05$. As can be seen by reference to individual trial data in Fig. 1, this effect was because of a more rapid acquisition by C75BL/6NNia mice treated with flumazenil. Analysis of variance and individual comparisons for latency to reach the safe platform revealed the same patterns as analyses of the number of avoidances.

DISCUSSION

A number of studies have provided evidence that improvement of learning and/or memory occurs following administration of compounds acting as antagonists or inverse agonists at benzodiazepine receptors. The antagonist compounds flumazenil (16), CGS-8216 (11), and ZK 93 426 (9,10), and ligands with inverse agonist activity such as Ro 15-4513 (13), FG 7142 (10) and β-CCM (30) have all been suggested to enhance cognitive performance in various experimental paradigms. The current finding, that flumazenil could improve active avoidance performance in two aging populations of mice, adds further support to the previous suggestion (16,25) that antagonist benzodiazepine ligands might be effective in reversal of aging-related cognitive impairment.

While the current findings are encouraging, several important issues must be addressed in future studies. The present experiment did not address which specific cognitive processes might be affected by the flumazenil pretreatments. Because our paradigm involved injection of drug prior to testing on each day, it is not clear whether the effects observed involve primarily acquisition, memory storage, or retrieval processes. Moreover, improvement of performance from day to day might have been the result of state-dependent learning processes [cf. (19)]. Previous investigations of younger mice suggested that both acquisition and memory storage were facilitated by flumazenil, and also indicated that state-dependent learning could not account for the effects observed (16).

In addition to the need for identifying the specific cognitive processes involved in the effects of flumazenil, the role played by affective processes needs to be clarified. While benzodiazepines could directly modify brain mechanisms of learning and/or memory, another likely possibility is that the mnemonic effects of flumazenil are secondary to modulation of anxiety or arousal states (20,31). Increased arousal can lead to enhanced learning/memory (18) and, thus, enhanced performance on the shock-motivated active avoidance paradigm used in the present study could be the result of enhanced anxiety or arousal following the flumazenil treatments.

Because the NZB/BlNJ mice show accelerated age-related declines in avoidance learning (3), the magnitude of avoidance impairments of the NZB/BlNJ mice would be expected to be much larger at 12–14 months when compared with the C57BL/6NNia mice. While the 12–14-month-old C57BL/6NNia mice did perform better than the NZB/BlNJ mice, previous studies have shown that performance of the 12–14-month-old C57BL/6NNia mice is impaired relative to younger C57BL/6NNia mice (3,4). Even though both strains show age-related avoidance impairments at the ages we tested, the present findings do not necessarily reflect a specific facilitation of those brain functions which are impaired during aging, since our previous investigations have shown that flumazenil can improve acquisition and retention in younger, "unimpaired" mice. However, the current findings do indicate that moderate brain aging does not prevent the performance-enhancing effects of flumazenil.

Because learning and memory facilitation could be obtained at relatively low doses of flumazenil or CGS 8216 (11,16), we previously postulated that the mnemonic effects of those drugs might involve antagonism of an endogenous ligand. Hypothetically, this ligand would be a diazepam-like agonist exerting a tonic inhibitory influence upon memory processes (11,16). Because inverse agonist-like properties have been reported for flumazenil and CGS 8216 at higher doses, however, inverse agonist activity could also account for the performance-enhancing effects of flumazenil in the current study (1, 7, 21). Although agonist-like effects of flumazenil have also been reported (7), is seems quite unlikely that such an effect could explain the current findings, since diazepam-like drugs would be expected to impair (not facilitate) learning or memory performance.

The mechanism whereby benzodiazepine ligands influence learning and memory processes may involve an interaction with the brain cholinergic system. Based upon known neuroanatomical relationships and findings that benzodiazepine antagonists could prevent scopolamine-induced amnesia, it has been suggested the effectors acting via the GABA-benzodiazepine receptor complex could inhibit activity of cholinergic neurons in brain areas important for learning/memory (16,25). Investigations of both normal mice [cf. (8)] and autoimmune NZB/BlNJ mice (4, 5, 22–24) have suggested that age-related learning/memory deficits could be at least partially attributed to compromised function of the cholinergic system, and therefore, reversal of age-associated learning/memory impairments may be related to disinhibition of the cholinergic system via the antagonist action of flumazenil.

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