The Effect of Age on the Behavioral Responses of Mice Following Diazepam and Midazolam Sedation in Combination with Nitrous Oxide

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This study examined the effects of age on the behavior of mice administered one of two benzodiazepines with and without nitrous oxide. Young (3 wk ± 3 days) and adolescent (7 wk ± 3 days) male DBA-2 mice were administered oral diazepam (2.0 or 3.5 mg/kg), midazolam (0.75 or 1.2 mg/kg), or a placebo in combination with 50% nitrous oxide/50% oxygen, or room air. The mouse staircase model was used, where the number of rears (NR) served as an index of anxiety, and the number of steps ascended (NSA) as an index of sedation. No significant differences in the responses between the ages were noted. Nitrous oxide seemed to increase the NR and NSA, whereas the benzodiazepines alone did not affect behavior. These DBA-2 mice may represent a strain that is less sensitive to the anxiolytic-sedative effects of the benzodiazepines than are other strains.

In the practice of pediatric dentistry, when nonpharmacologic approaches to behavior management have been unsuccessful, pharmacologic management is indicated. One population that would benefit from the antianxiety and sedative effects of premedication are younger patients who, due to their age, are not able to cooperate. There have been few studies of drugs and dosages in young subjects, probably due to inherent difficulties in studying this population. Similarly, the literature is sparse in studies using a young animal model. Therefore, further research in this area, particularly in the establishment of an appropriate animal model for sedation, is needed.

The purpose of this study was to examine, using a behavioral model, the effects of age on the sedative and antianxiety effects in mice administered diazepam or midazolam with and without nitrous oxide.

METHODS

Animals

One hundred and twenty DBA-2 inbred mice (Jackson Laboratory, Bar Harbor, MA and Charles River Laboratory, No. Wilmington, MA), half 3 wk old ± 3 days (young) and half 7 wk old ± 3 days (adolescent) were used in this investigation. The weaning age of the mouse (3 wk) was used to represent childhood, and breeding age (7 wk) was used to represent adolescence.

Apparatus

The experimental testing was conducted in a clear plexiglass chamber (100 L in volume). An inflow port at the top was fitted to accept nitrous oxide and oxygen from an analgesia delivery machine or air, which was supplied in the laboratory. The outflow tube at the bottom was attached to a suction device that removed excess gas and maintained a constant internal pressure within the testing chamber. A hole in the front of the chamber was fitted with a neoprene accordion sleeve to permit investigator access to the testing area without allowing the escape of air or gas mixture.

The chamber was compartmentalized into two sections: a holding area used during the acclimation period, and a testing area where the behavioral testing was performed.

Behavioral Testing

The behavioral testing was conducted using the mouse staircase test. A white, plastic, five-step staircase was fabricated in accordance with the specifications of Simiand et

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al.[1] The number of steps ascended (NSA) and the number of rears (NR) exhibited were counted and recorded over a 3-min testing period. NSA was used as an index of locomotion/sedation, and the NR was used as an index of anxiety. Each mouse was tested only once in order to eliminate possible effects of learning and familiarization. Following each behavioral testing period, the staircase was cleaned with distilled water to eliminate any olfactory cues.

Agents

The drugs used in this investigation were diazepam (2.0 mg/kg and 3.5 mg/kg, Sigma Chemical Co., St. Louis, MO) and midazolam (0.75 mg/kg and 1.2 mg/kg, donated by Hoffman-La Roche, Inc., Nutley, NJ). The benzodiazepines were suspended in an aqueous suspension of maanide monooleate (Arlacel A, Sigma), which also served as the placebo. Nitrous oxide was admitted into the testing chamber in a 50% concentration with 50% oxygen.

Procedure

The experiment was conducted using a randomized group design. On each of four test sessions, both age groups and all five drug treatment groups (diazepam low and high dose, midazolam low and high dose, and placebo) were represented. A single concentration of nitrous oxide (0% or 50%) was randomly assigned to each session yielding two sessions with and two sessions without nitrous oxide. Thirty naive mice (15 young and 15 adolescent) were each assigned to a drug treatment according to a randomization table prepared for that particular session, resulting in six mice for each group after all sessions were completed. The drugs were administered orally using a straight, 24-ga epigastric feeding needle. The volume of drug suspension administered was 1.5% of the total body weight. Drug concentrations were adjusted to allow for standard volumes. Following a 45-min waiting period for the absorption of the medication, the mice were admitted into the holding area in the testing chamber for an additional 15-min acclimation period in either the nitrous oxide or room air environment. The investigator/observer was blinded as to the type of drug treatment.

This protocol was approved by the Institutional Animal Care and Use Committee of the UMDNJ-New Jersey Medical School.

Statistical Analysis

Mean scores ± standard errors for NR and NSA were calculated for each experimental testing group. The 95% level of confidence was also determined. A one-way analysis of variance (ANOVA) was used to determine the statistical significance among the 10 treatment groups; Student's *t*-test was used to determine differences between the ages of the mice (young versus old). Dunnett's test and Bonferroni's multiple comparison tests were used after the ANOVA. The 5% level of probability was used throughout.

RESULTS

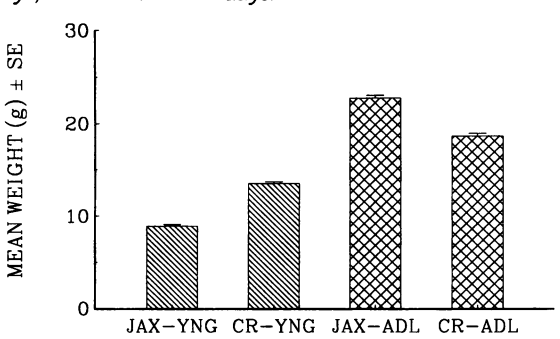
The original animal vendor, Jackson Laboratory, was unable to supply the full complement of mice towards the middle of the investigation. Therefore, a second batch of the identical strain was obtained from the Charles River Laboratory. Upon receipt of the animals, weight differences within each age group were discovered between the Jackson and Charles River Laboratories (Figure 1). The young mice from the Charles River Laboratory were 51% heavier than the young Jackson Laboratory mice ($t = 12.45$, $P < 0.05$). On the other hand, the adolescent Charles River mice were lighter than the Jackson mice by 18% ($t = 8.65$, $P < 0.05$). Both vendors confirmed the correctness of the ages of the mice.

Upon completion of the behavioral testing, the responses (NSA and NR) of the mice from the Jackson and Charles River Laboratories, within each age group, among the respective treatments, were compared using Student's *t*-test. In 85% of the treatment groups no significant differences were noted between the mice from the two vendors, and the responses of the mice from the two vendors were then pooled.

NR - Young Mice

The NR exhibited by the young mice is represented in Figure 2. Dunnett's test was used to compare the control (placebo in room air; P-AIR) with each of the other nine

Figure 1. Weights of two age groups of mice from two different vendors (n = 30 mice per age-vendor group). JAX = Jackson Laboratory; CR = Charles River Laboratory; YNG = 3 wk ± 3 days; ADL = 7 wk ± days.



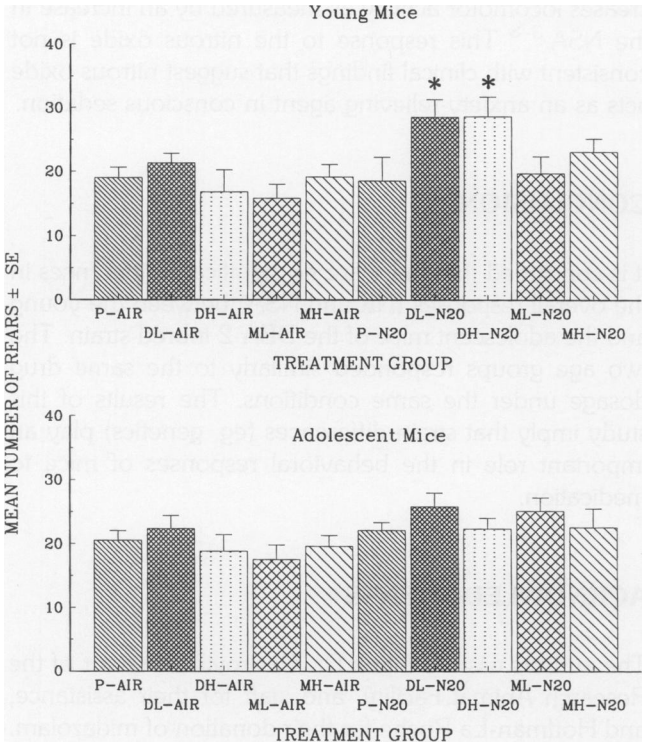


Figure 2. Mean NR exhibited by young and adolescent mice following the administration of 10 different drug treatments (6 mice per group). Dunnett's test (*$P < 0.05$) was used to compare the standard (placebo drug in room air, P-AIR) with each of the nine remaining treatments. P, = placebo; DL = diazepam, low dose; DH = diazepam, high dose; ML = midazolam, low dose; MH = midazolam, high dose; AIR = room air; N_2O = nitrous oxide.

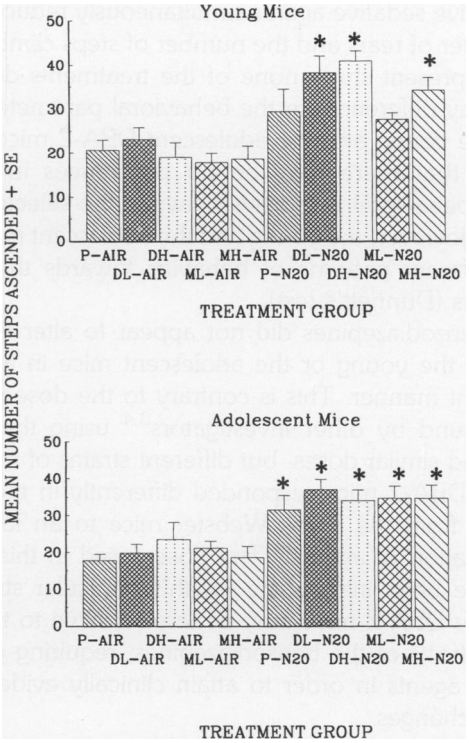


Figure 3. Mean number of steps ascended exhibited by the young and adolescent mice following the administration of 10 different drug treatments (6 mice per group). Dunnett's test (*$P < 0.05$) was used to compare the standard (placebo drug in room air; P-AIR), with each of the nine remaining treatments. P = placebo; DL = diazepam, low dose; DH = diazepam, high dose; ML = midazolam, low dose; MH = midazolam, high dose; AIR = room air; N_2O = nitrous oxide.

treatments. The only treatments significantly different from the control were the two doses of diazepam administered in combination with nitrous oxide, where the NR was increased by 33%.

NR - Adolescent Mice

In contrast to the differences in the number of rearings responses in young mice, (Figure 2) shows that none of the rearing responses to the nine treatments administered to the adolescent mice were different from the control.

NSA - Young Mice

Figure 3 illustrates the NSA by the young mice. Three of the four benzodiazepine treatments in the nitrous oxide environment increased the number of steps climbed by an average of 44%. The low dose of midazolam, together with nitrous oxide, was not statistically different from the control (Dunnett's test).

NSA - Adolescent Mice

The step-climbing activity in the adolescent DBA-2 mice is revealed in Figure 3. All treatments in the nitrous oxide environment significantly increased the NSA by an average of 43% to 51% when compared with controls (Dunnett's test).

Upon completion of the behavioral test, the NR and NSA performed by the young and the adolescent mice were compared using Student's *t*-test to determine if there were any differences in the responses between the two age groups. No significant differences were found.

DISCUSSION

The staircase test in mice has been shown to be simple, rapid, economical, and selective for anxiolytic drugs, particularly the benzodiazepines. Conventional anxiolytics have been reported to decrease rearings at doses that do not reduce the number of steps ascended, whereas the

nonselective sedative agents simultaneously reduce both the number of rears and the number of steps climbed.[3,4]

In the present study none of the treatments demonstrated any differences in the behavioral parameters between the young and the adolescent DBA-2 mice. This suggests that there may be no differences in drug-induced behavioral responses between the selected age groups. However, the young and the adolescent mice did show different patterns of behavior towards the drug treatments (Dunnett's test).

The benzodiazepines did not appear to alter the behavior of the young or the adolescent mice in a dose-dependent manner. This is contrary to the dose-related effects found by other investigators[3,4] using the same model and similar doses, but different strains of mice. In fact, the DBA-2 mice responded differently in the staircase test from the Swiss Webster mice to an identical administration of agents.[5] The doses used in this study may have been inadequate for this particular strain of mice. The DBA-2 mice may be less sensitive to the anxiolytic effects of the benzodiazepines, requiring greater doses of agents in order to attain clinically evident behavioral changes.

An intriguing finding of the behavioral responses to diazepam in the staircase model was revealed by Steru, et al.[2] Using a wide range of doses of 11 different benzodiazepines in mice, this investigator found a biphasic response in the NR and NSA. The dose-response curves produced were in the form of an inverted U, an initial increase in physical activity followed by a decrease in both parameters at higher doses. The earlier work by Simiand, et al[1] employed doses at the middle to high range and produced a dose-response curve similar to the descending portion (higher dose range) of the Steru, et al[2] curve. This was confirmed by Pruhs and Quock[4] who used a comparable dose range to that of Simiand, et al.[1]

Steru, et al[2] made a significant clarification in the understanding of this model because the use of a wide range of doses allowed for the expression of a biphasic response in both the NSA and NR.

The DBA-2 mice seem to respond similarly to those mice administered a low dose of diazepam in the Steru study.[2] This result suggests that the subjects used in the present study may be less sensitive to the effects of the benzodiazepines when compared with other strains.

Nitrous oxide (50%) seemed to have a greater effect on the behavior of the mice than either the midazolam or diazepam. Nitrous oxide, with or without a benzodiazepine, produced a significant increase in the NSA without any consistent effect on the NR. Previous studies using nitrous oxide have also shown that its administration increases locomotor activity as measured by an increase in the NSA.[3–8] This response to the nitrous oxide is not consistent with clinical findings that suggest nitrous oxide acts as an anxiety-relieving agent in conscious sedation.

CONCLUSION

It is concluded that there are no significant differences in the overall responses (NR and NSA) between the young and the adolescent mice of the DBA-2 inbred strain. The two age groups responded similarly to the same drug dosage under the same conditions. The results of this study imply that strain differences (eg, genetics) play an important role in the behavioral responses of mice to medication.

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