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Acute Tolerance and Reversal of the Motor Control Effects of Midazolam

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COLDWELL, S. E., E. KAUFMAN, P. MILGROM, E. D. KHARASCH, P. CHEN, D. MAUTZ AND D. S. RAMSAY. *Acute tolerance and reversal of the motor control effects of midazolam.* PHARMACOL BIOCHEM BEHAV **59**(2) 537–545, 1998.—The purpose of this study was to determine whether acute tolerance develops to the motor control effects of the short-acting benzodiazepine, midazolam. Using a bolus and constant infusion scheme, 40 healthy adults received a 70-min intravenous infusion of either saline (n = 20) or 6.1 (SE = 0.2) mg midazolam (n = 20). Following the 70-min infusion period, half of the subjects in each group (n = 10) received a 25-min intravenous infusion of flumazenil (benzodiazepine antagonist); the remainder of the subjects (n = 10/group) received a 25-min infusion of saline. Drug administration during both infusion periods was double blind. Prior to the infusions, subjects were trained in a motor control assessment battery. Throughout both infusions, repeated motor control testing and blood sampling were performed. The initial (10 min) midazolam plasma concentration was 52.0 (SE = 2.2) ng/ml. Plasma midazolam concentration rose gradually to 60.7 (SE = 2.1) ng/ml at the end of the infusion (70 min). Midazolam initially impaired performance on the motor control tasks. However, performance improved in subjects receiving midazolam despite the gradual increase in midazolam concentrations. This suggests that the recovery of motor task performance may be attributable to the development of acute tolerance rather than to waning drug concentrations. Flumazenil immediately reversed midazolam's effects on the visual tracking task. However, there was little evidence for precipitation of muscle force rebound, which has been hypothesized to result from the same underlying mechanism that is responsible for acute tolerance development. © 1998 Elsevier Science Inc.

Flumazenil Rebound Tachyphylaxis

THE benzodiazepine class of drugs has sedative, anxiolytic, and CNS-mediated muscle-relaxant effects (20). These properties have led to their wide-spread use for alleviating anxiety and insomnia (15). However, chronic administration of benzodiazepines has been found to result in the development of behavioral tolerance to the drug's effects, and to be associated with withdrawal syndromes upon discontinuation of use (15,27,38). For example, rebound increases (above pretreatment levels) of anxiety and insomnia have been frequently reported upon cessation of chronic benzodiazepine use (8,21,39). Muscle effects, such as muscle cramps and muscle fasciculations, have also been documented (38). Similarly, rebound increases in subjective symptoms have been precipitated by the administration of the benzodiazepine antagonist, flumazenil, following chronic benzodiazepine administration (14,41,48).

Acute tolerance to the sedative and psychomotor effects of the benzodiazepines has also been reported following short-term benzodiazepine administration (10,11,26,44). However, little research has been done to investigate whether rebound effects accompany the development of tolerance during the course of a single benzodiazepine administration. Such rebound effects would be predicted by a number of theoretical models, which suggest that tolerance and rebound are produced by a common compensatory process (16,25,35,36).

The purpose of this investigation was to determine whether acute tolerance develops to the motor control effects of the short-acting benzodiazepine, midazolam, in humans. We also wished to determine whether compensatory changes in muscle force may account for this tolerance. The presence of compensatory increases in muscle force was evaluated by

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determining whether a rebound effect could be precipitated by the administration of flumazenil (31). Assessing the development of acute tolerance is facilitated by controlling blood concentrations of the drug (27,30). This was accomplished by assessing the motor control effects of midazolam during an infusion paradigm designed to approximate constant plasma drug concentrations. The motor control effects of midazolam were assessed using a battery of computer-controlled tests that measures the ability to produce, monitor, and control muscle force (23,29). Thus, this study was designed to determine 1) whether the motor control effects of midazolam would impair subjects' performance on a behavioral motor control battery, 2) whether acute tolerance to the motor control effects of midazolam would develop, and 3) whether rebound increases in force would be observed when the action of midazolam was abruptly antagonized by flumazenil.

METHOD

Subjects

Forty subjects (20 male, 20 female) were recruited by advertisement. The average age and weight were 27 years (range 19 to 44 years) and 68.8 (±2.2) kg, respectively. None of the subjects had used sedative drugs within the previous year, and none were currently taking any prescription drugs other than oral contraceptives. All subjects denied alcohol or substance abuse, were not under psychiatric care, and had no contraindications for the use of midazolam (e.g., glaucoma, pregnancy). This study was approved by the human subjects review committee at the University of Washington. Written informed consent was obtained from all subjects.

Apparatus

Motor control data were collected using a hand-held force transducer (Interface, Inc., Scottsdale, AZ). The analog signal from the force transducer was digitized using an analog to digital converter on a multifunction card (National Instruments, Austin, TX) operating in an Apple Macintosh IIci personal computer. Force data were saved every 0.25 s during the force tasks (described below). The LabVIEW programming language (National Instruments, Austin, TX) was used for experimental control as well as data acquisition and analysis.

Procedures

Drug administration. A loading dose of midazolam (0.035 mg/kg) was delivered over 2 min, followed by a constant infusion of 0.75 μg/kg/min for 70 min using a Baxter AS20GH infusion pump. Midazolam concentration was targeted to lie between 50 and 75 ng/ml. This dose of midazolam was selected based on pilot work, which revealed a large drug effect on the motor control tasks within these concentrations. A loading dose of flumazenil (0.030 mg/kg) was delivered over 2 min, followed by a constant infusion of 0.32 μg/kg/min for 25 min. Flumazenil concentration was targeted at 25 ng/ml. Saline placebo was delivered in the same manner as the active drugs. Drug assignment was double blind.

Isometric force tasks. The behavioral test battery for motor control was modified from that developed by Mai and colleagues (23,29). This battery was designed to assess muscle strength, as well as the ability to maintain a constant level of force. During testing, except where otherwise noted, visual feedback from the force generated by the subject was plotted against elapsed time on a computer monitor placed in front of the subject.

The maximum force task was used to measure muscle strength. Following an attention beep, subjects were given 5 s to squeeze the hand-held transducer as strongly as possible. To minimize fatigue, subjects were advised to squeeze up to a maximum peak and then to release the transducer. The maximum force measurable by the apparatus was 15.5 kg. (While kg is a unit of mass and not force, the force transducer was calibrated using kg weights; therefore, we have chosen to report the findings in kg units. Using the idealized standard value of gravitational acceleration near sea level of 9.80 meters/s squared, 1 kg-mass × 9.80 m/s^2 = 9.80 newtons.)

The visual tracking task required subjects to maintain a constant force of 1.75 kg. The target force was set at 1.75 kg because this force is easily generated by most subjects, resulting in reduced muscle fatigue (Beth Kerr, personal communication). A horizontal line representing a force of 1.75 kg appeared across the center of the subject's monitor. Visual feedback from the force generated by the subject was also plotted against elapsed time on the screen. Subjects were given the opportunity to correct errors in generated force because the visual feedback indicated any discrepancy between their performance and the target force value. Subjects were instructed to adjust their squeezing strength on the transducer to track the center target line as closely as possible. This task was performed for 20 s. An attention beep indicated the beginning and end of the 20-s period.

The hidden tracking task required subjects to maintain a constant force of 1.75 kg. As in the visual tracking task, a horizontal line representing a force of 1.75 kg appeared across the center of the subject's monitor. However, subjects were given visual feedback only during the first half of the task. Visual feedback indicating the force generated by the subject was, therefore, visible on the screen only during the first 10 s of the task. Subjects were instructed to produce sufficient force to stay on the center target line, and to continue doing so for the final 10 s of the task after visual feedback had disappeared. An attention beep indicated the beginning and end of the 20-s period of the task.

Experimental Protocol

Subjects were tested individually in an enclosed dental operatory. Each subject was seated in a dental chair, with his (her) dominant arm extended and resting on a foam platform. Prior to each force task the experimenter placed the transducer into the subject's dominant hand. Subjects were instructed to support the transducer between the index and middle fingers and thumb. Subjects received visual feedback from each task on a 32-cm monitor, which was placed on a tray positioned approximately 75 cm directly in front of the face. Instructions to the experimenter were provided on a second 32-cm monitor, which was out of the view of the subject.

Subjects refrained from food and liquids for 6 h prior to testing. Subjects were given a 1-h practice session with the force tasks to minimize changes in performance due to practice. During the practice session, each subject performed six sets of force assessments. Each force assessment set consisted of one maximum force task, two visual tracking tasks, and two hidden tracking tasks. Each assessment took 2–5 min to complete. The assessments were initiated at 10-min intervals.

Following the practice session, each subject was given a 1-h rest. Afterwards, the subject was reseated in the dental chair, physiological monitors were attached, and a 20 g catheter was placed in the antecubital vein of each arm. One catheter was used for drug administration, and the other catheter was used

for blood sampling. Following catheter placement, each subject repeated the motor control battery twice in the same manner as in training. Performance on these two occasions was used to establish baseline (preinfusion) performance for each task. A baseline blood sample (8 ml) was also taken immediately following these assessments.

Subjects were randomly assigned to receive midazolam or saline during the first infusion period and flumazenil or saline in the second infusion period (Table 1). Gender was balanced across all drug conditions. Both subject and experimenter were blinded as to the drug treatment. Midazolam or placebo was infused into one arm (in most cases, the dominant arm), while blood was drawn from the opposite arm. Throughout the drug and placebo infusions, heart rate and blood oxygen saturation were continuously monitored. Blood pressure was automatically taken every 5 min during the two infusion periods.

Once the baseline blood sample had been obtained, subjects received a midazolam or saline bolus and continuous infusion over a 70-min period according to the parameters described above. At 70 min, the midazolam or saline infusion was terminated. The flumazenil or saline bolus and infusion were begun 5 min later. This second infusion period lasted for 25 min. Behavioral assessments of motor control were conducted throughout the infusion periods as in training. The first assessment was conducted 5 min after the start of the first infusion period. [The half-life for equilibrium between plasma and brain for midazolam has been estimated to be between 1.7 and 4.8 min (5–7,33)]. Blood (8 ml) was collected at 10, 30, 50, 70, 85, and 105 min relative to the start of the first infusion period. Blood samples were immediately transferred to centrifuge tubes, mixed with EDTA, and centrifuged for 7–10 min. The plasma was frozen at −70°C for later analysis. Following withdrawal of the final blood sample, catheters, and monitoring equipment were removed. Each subject was checked to assure that he/she had good ambulatory function before being discharged to an escort, who provided transportation home.

Plasma midazolam concentration. Culture tubes (13 × 100 mm) with internal standard (diazepam, 30 ng in 30 μl of MeOH) received 1 ml of plasma and 1 ml of 50 mM carbonate buffer. The samples were mixed with 19:1 toluene:isoamyl alcohol by a vigorous vortex shaker, and the emulsions were then broken by 3 min of centrifugation at 1400 g. The upper liquid phases were transferred to new culture tubes (13 × 100 mm) and the organic layer was reduced to dryness by a gentle nitrogen stream. Each residue was dissolved in 50 μl of methanol and was transferred to autosampler vials with reduced volume inserts for analysis. Midazolam was detected and quantified by gas chromatography using a Hewlett-Packard Model 5890 II gas chromatograph/electron capture detector with a DB-17 (30 m × 0.32 mm × 0.25 μm) capillary column (J & W, Folsam, CA). Injections of 1 μl were made in a spitless mode with an EPC controlled pressure pulse of 30 psi of the helium carrier gas. After 1 min, the head pressure was decreased to 5 psi and the inlet purged. Injector and transfer line temperatures were 260 and 300°C, respectively. The oven temperature program was held at an initial 40°C temperature for 1 min then increased to 250°C at 30°C/min, to 280°C at 10°C/min, to 300°C at 5°C/min and held at 300°C for 1 min. Midazolam and diazepam retention times were 9.85 and 8.57 min, respectively. Standard curves were constructed using blank plasma spiked with appropriate midazolam concentrations, treated identically to patient samples, and used for determination of midazolam concentrations in patient samples using peak area ratios. The interday coefficient of variation was 6% for 50 ng/ml midazolam quality control samples ($n = 6$).

Data Analysis

Plasma midazolam concentration. Midazolam concentrations during the midazolam infusion were analyzed using ANOVA with time of blood sample (10, 30, 50, or 70 min postinfusion) as a within-subjects factor. Because ovarian hormones have been suggested to influence both metabolism of and chronic tolerance to the benzodiazepine diazepam (1,47), an additional analysis of plasma midazolam concentration was conducted with gender as a between-subject factor and time as a within-subject factor.

Force tasks. For the maximum force task, the peak force generated over the 5-s testing period was obtained for each assessment. For the visual tracking and hidden tracking tasks all signed deviations from the 1.75-kg target value were added to yield a summed error score [Kerr and colleagues (23) refer to this measure as constant error]. Negative sums indicate undershooting of the target, while positive sums indicate overshooting. This analysis method permits the detection of any change in direction of error from baseline to experimental condition, but does not detect absolute magnitude of error. This analysis method was selected to permit the detection of the hypothesized decrease (drug effect) and subsequent increase (rebound) in muscle force. [Analyses of absolute magnitude of error (23) were also conducted. These analyses yielded essentially the same pattern of data. However, the magnitude of the drug effect appeared somewhat smaller, and variability was larger, using this measure. Therefore, these analyses will not be discussed further.] Deviations were summed across the entire 20 s of the visual tracking task. The deviations were summed only across the final 10 s (hidden part) of the hidden tracking task. The scores obtained on the two visual tracking tasks and the scores obtained on the two hidden tracking tasks of each assessment were averaged to yield one score for each task at each assessment. These performance scores at each assessment period were then subtracted from baseline performance. The first and second infusion periods were analyzed separately. Each task was also analyzed separately.

TABLE 1
ASSIGNMENT OF SUBJECTS TO MIDAZOLAM/PLACEBO AND FLUMAZENIL/PLACEBO CONDITIONS

Group	Number and Gender of Subjects (M or F)	Mean Weight in kg (SE)	Mean Age in years (SE)	First Infusion (minute 0–70)	Second Infusion (minute 75–100)
MF	4 M, 5 F	64.4 (± 3.8)	25 (± 1)	Midazolam	Flumazenil
MP	5 M, 5 F	73.7 (± 3.4)	28 (± 2)	Midazolam	Placebo
PF	5 M, 5 F	70.4 (± 5.9)	26 (± 2)	Placebo	Flumazenil
PP	5 M, 5 F	64.1 (± 3.9)	27 (± 3)	Placebo	Placebo

For each task, data collected during the first infusion period were analyzed using a 2 × 7 ANOVA. The ANOVA used group (placebo or midazolam) as a between-subject factor and assessment time as a within-subject factor. When ANOVA yielded a significant group by time interaction, preplanned tests for drug effect and acute tolerance were conducted. Drug effect was tested by comparing midazolam and saline-infused subjects at both the first (5-min) and last (65-min) assessments. Acute tolerance was tested by comparing the first and last assessments of the infusion period for subjects given midazolam.

Data collected during the second infusion period were analyzed using 4 × 3 ANOVAs, with group (midazolam-placebo, midazolam-flumazenil, placebo-placebo, or placebo-flumazenil) as a between-subject factor and assessment time as a within-subject factor. When ANOVA revealed a main effect of group, three preplanned comparisons were made: between group midazolam-flumazenil and group placebo-flumazenil (precipitated rebound); between group midazolam-placebo and group placebo-placebo (rebound); and between group midazolam-flumazenil and group midazolam-placebo (flumazenil's antagonist effect). These preplanned comparisons were collapsed across time of assessment. The criterion for statistical significance for all tests was $p < 0.05$. All analyses were performed using Statistica (version 4.3) software (StatSoft, Tulsa, OK).

RESULTS

Many subjects appeared sedated during the midazolam infusion, although all subjects readily responded to verbal commands. However, one (39 year-old, 89.8 kg male) subject in the midazolam-flumazenil condition was not able to remain awake during the first infusion period, and was too sedated to perform the motor control tasks. The data for this subject are not included in the analyses.

Plasma Levels

Midazolam-infused subjects received a total dose of 6.1 ± (SE) 0.2 mg midazolam. Flumazenil-infused subjects received a total dose of 2.7 ± 0.1 mg flumazenil. For subjects receiving midazolam, plasma midazolam levels rose to an initial mean level of 52.0 ± 2.2 ng/ml and then increased slightly to 60.7 ± 2.1 ng/ml over the 70-min infusion period, $F(3, 54) = 11.1$, $p < 0.001$. Hence, we achieved a reasonably stable level of drug throughout the infusion period. Plasma midazolam concentrations for women averaged 6 to 12 ng/ml lower than those for men, $F(1, 17) = 9.84$, $p < 0.01$ (Fig. 1). Women also weighed less than the men, 58.5 ± 1.68 kg vs. 78.6 ± 2.5 kg, $F(1, 37) = 43.4$, $p < 0.0001$. The gender difference in plasma midazolam concentration was consistent across all six sampling times [gender by time interaction, $F(5, 85) = 0.69$, $p = 0.63$].

Maximum Force

First infusion period. Baseline maximum force was 9.56 ± 0.87 kg for group placebo-flumazenil, 10.90 ± 1.09 kg for group placebo-placebo, 10.29 ± 1.05 kg for group midazolam-flumazenil, and 11.52 ± 0.92 kg for group midazolam-placebo. Baseline maximum force values did not significantly differ among groups, $F(3, 35) = 0.69$, $p = 0.56$. Five minutes after initiating drug infusion, subjects receiving midazolam produced lower peak muscle force (relative to baseline scores) than those receiving saline, $F(1, 37) = 6.41$, $p < 0.02$ (Fig. 2). However, subjects receiving midazolam rapidly became tolerant to these effects, such that no differences between subjects receiving midazolam and saline were found 65 min into the infusion period. Peak force was significantly higher at 65 min than at 5 min for subjects receiving midazolam, $F(1, 37) = 9.11$, $p < 0.01$. Peak force did not change over the infusion period for subjects receiving saline.

Second infusion period. All four groups improved from the 80-min to the 100-min assessment $F(2, 70) = 5.84$, $p < 0.005$. There were no significant differences among groups in peak force scores throughout the second infusion.

Visual Tracking

First infusion period. Baseline constant error for visual tracking was −3.25 ± 0.65 kg for group placebo-flumazenil, −2.97 ± 0.77 kg for group placebo-placebo, −2.81 ± 1.07 kg for group midazolam-flumazenil, and −2.65 ± 0.84 kg for group midazolam-placebo. Baseline constant error values for visual tracking did not significantly differ among groups, $F(3, 35) = 0.96$, $p = 0.42$. Throughout the first infusion period, subjects receiving midazolam undershot the target (relative to baseline performance) more than did subjects receiving saline, $F(1, 37) = 22.8$, $p < 0.001$ (Fig. 3). Significant differences

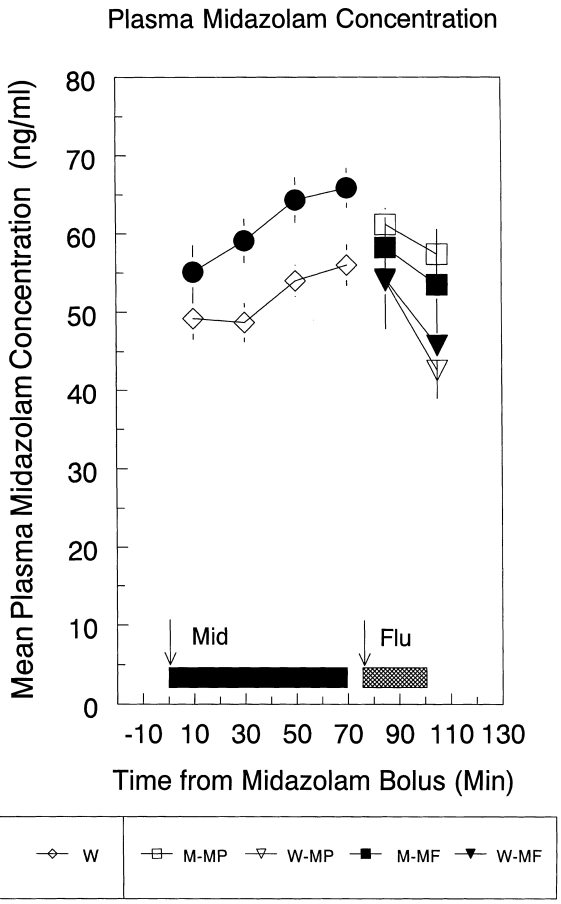

FIG. 1. Mean plasma midazolam concentrations (± standard error) are depicted for men ($n = 9$, dark circles) and women ($n = 10$, open diamonds) receiving midazolam during a 70-min infusion of midazolam ("Mid," black bar). Also depicted are plasma midazolam concentrations for men (squares) and women (triangles) who subsequently received a 25-min infusion ("Flu," stippled bar) of either flumazenil (filled symbols) or saline placebo (open symbols).

among groups were present at both the first and last assessment periods, $F(1, 37) = 16.2$, $p < 0.001$, and $F(1, 37) = 12.0$, $p < 0.01$, respectively. However, subjects receiving midazolam improved from the first to the last assessment, $F(1, 37) = 13.0$, $p < 0.001$. There was no significant improvement from the first to the last assessment for subjects receiving saline, $F(1, 37) = 0.20$, $p = 0.66$.

Second infusion period. During the second infusion period, group midazolam-flumazenil performed better on the visual tracking task than did group midazolam-placebo, indicating that flumazenil enhanced recovery, $F(1, 35) = 7.48$, $p < 0.001$. However, there was no indication that flumazenil produced rebound in midazolam-treated subjects (i.e., no differences were found between group midazolam-flumazenil and group placebo-flumazenil, Fig. 3).

Hidden Tracking

First infusion period. Baseline constant error was -2.27 ± 0.73 kg for group placebo-flumazenil, -0.07 ± 0.50 kg for group placebo-placebo, 0.28 ± 0.76 kg for group midazolam-flumazenil, and -0.60 ± 0.81 kg for group midazolam-placebo. Baseline constant error values for hidden tracking did not significantly differ among groups, $F(3, 35) = 2.55$, $p = 0.07$. When visual feedback was unavailable, subjects receiving midazolam undershot the target (relative to baseline performance) more than did subjects receiving saline, $F(1, 37) = 5.43$, $p < 0.03$ (Fig. 4). There was no group by time interaction to indicate that this effect changed differentially over time for the two groups.

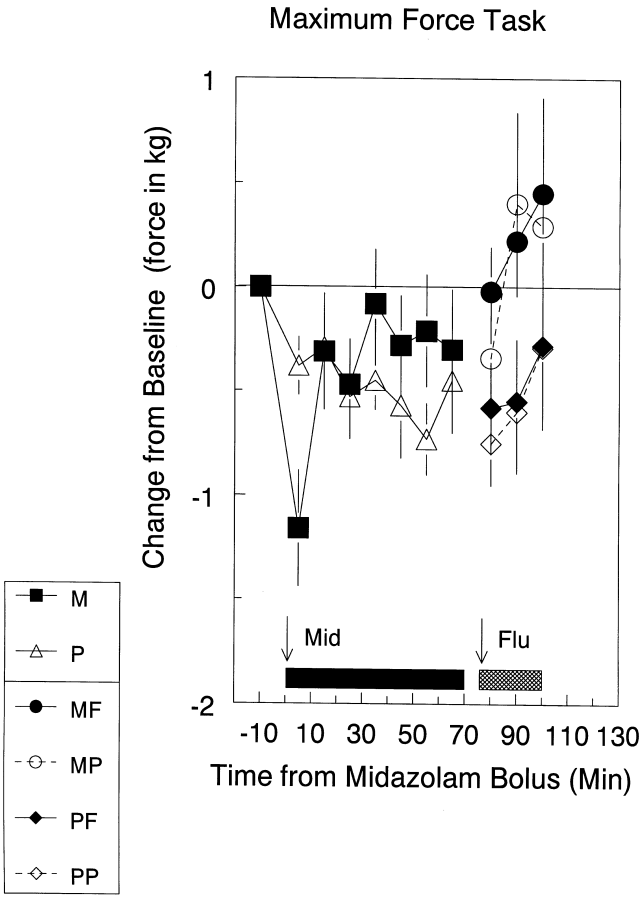

FIG. 2. Performance on the maximum force task during the infusion periods is depicted as change from baseline performance. Negative values indicate that less force was produced than at baseline. Positive values indicate that more force was produced than at baseline. In the first infusion period subjects were infused for 70 min with either midazolam ($n = 19$, filled squares) or saline placebo ($n = 20$, open triangles). In a subsequent 25-min infusion period, 9 subjects in the midazolam condition (filled circles) and 10 subjects in the placebo condition (filled diamonds) were administered flumazenil. The remaining 10 subjects in the midazolam condition (open circles) and 10 subjects in the placebo condition (open diamonds) were administered saline placebo in the second infusion period. Values shown are means ± standard error of the mean. The time period of the midazolam/placebo infusion is indicated by a black bar. The time period of the flumazenil/placebo period is indicated by the stippled bar.

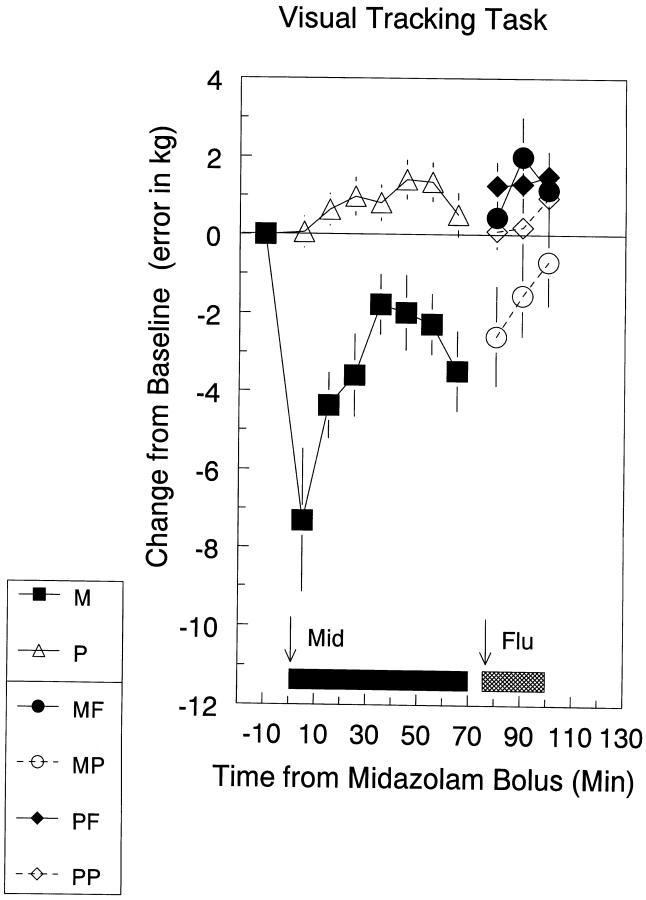

FIG. 3. Performance on the visual tracking task during the infusion periods is depicted as change from baseline performance. Negative values indicate that the target force (1.75 kg) was undershot in comparison with baseline performance. Positive values indicate that the target force was overshot in comparison with baseline performance. In the first infusion period subjects were infused for 70 min with either midazolam ($n = 19$, filled squares) or saline placebo ($n = 20$, open triangles). In a subsequent 25-min infusion period, 9 subjects in the midazolam condition (filled circles) and 10 subjects in the placebo condition (filled diamonds) were administered flumazenil. The remaining ten subjects in the midazolam condition (open circles) and 10 subjects in the placebo condition (open diamonds) were administered saline placebo in the second infusion period. Values shown are means ± standard error of the mean. The time period of the midazolam/placebo infusion is indicated by a black bar. The time period of the flumazenil/placebo period is indicated by the stippled bar.

Second infusion period. No significant group differences were revealed during the second infusion period for the hidden tracking task.

Gender

Because plasma midazolam concentrations differed by gender, separate analyses of the force task data were conducted with gender included as a factor. No statistically significant effects of gender were found for any of the force tasks.

DISCUSSION

Midazolam produced deficits in all three motor control tasks assessed. The observed impairments were a decrease in maximum force and an undershooting of targeted force, which is consistent with midazolam's CNS-mediated muscle relaxation effects (34). However, performance for the maximum force and visual tracking tasks improved over the course of the midazolam infusion, even though plasma midazolam concentrations increased slightly. Although significant improvement was not detected in the hidden tracking task, the failure to observe such an effect is likely due to the large variability in performance on the task, rather than a clear absence of improvement. (Large variability in hidden tracking task performance is evident even at baseline.) These results suggest that subjects developed acute tolerance to the effects of midazolam within the brief infusion period employed here, and in spite of the fact that midazolam levels in the plasma actually increased slightly during the infusion. There was no evidence for an effect of gender in the development of acute tolerance to midazolam. Despite the development of tolerance, statistically significant rebound increases in muscle force were not observed when flumazenil was administered to midazolam-tolerant subjects. Such an increase in muscle force performance would have been an indication that compensatory increases in muscle force were developing in parallel with the development of acute tolerance in subjects receiving midazolam.

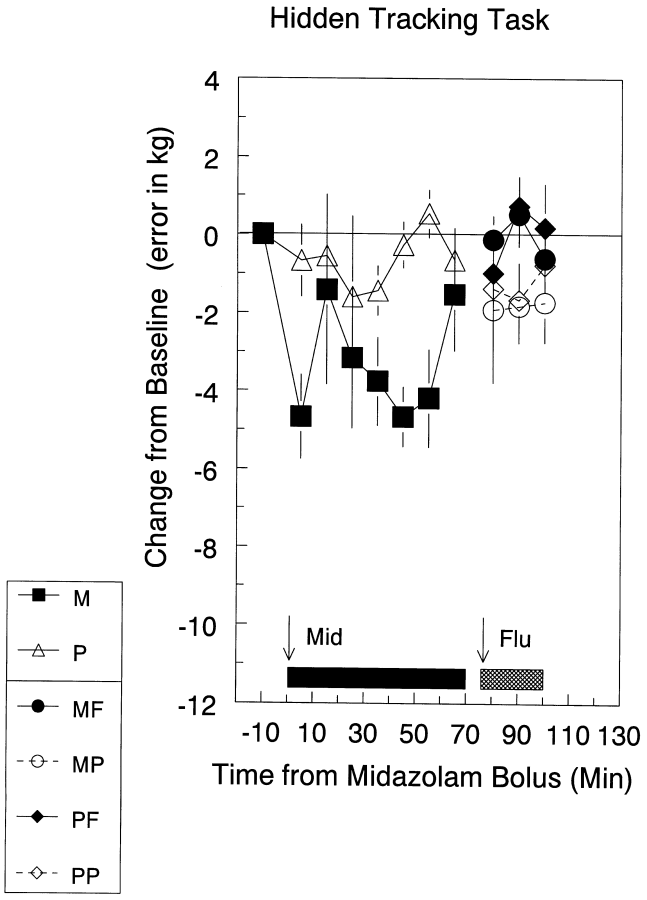

FIG. 4. Performance on the hidden tracking task during the infusion periods is depicted as change from baseline performance. Negative values indicate that the target force (1.75 kg) was undershot in comparison with baseline performance. Positive values indicate that the target force was overshot in comparison with baseline performance. In the first infusion period subjects were infused for 70 min with either midazolam (n = 19, filled squares) or saline placebo (n = 20, open triangles). In a subsequent 25-min infusion period, 9 subjects in the midazolam condition (filled circles) and 10 subjects in the placebo condition (filled diamonds) were administered flumazenil. The remaining 10 subjects in the midazolam condition (open circles) and 10 subjects in the placebo condition (open diamonds) were administered saline placebo in the second infusion period. Values shown are means ± standard error of the mean. The time period of the midazolam/placebo infusion is indicated by a black bar. The time period of the flumazenil/placebo period is indicated by the stippled bar.

In general the short-acting benzodiazepines, such as midazolam, have been found to produce more tolerance, dependence, and withdrawal symptoms than the longer acting benzodiazepines (17,38). Consistent with this, both acute and chronic tolerance to midazolam have been observed in animals and humans (2,18,21,24,41,45). However, many studies have failed to observe tolerance to midazolam in human subjects (3,28,32,42,44).

Failures to observe acute tolerance to midazolam may in part be due to the common use of crossover designs, which permit carryover effects from previous benzodiazepine usage to obscure observations of acute tolerance [e.g., (42,44)]. In the present investigation, subjects were exposed to midazolam only once, and subjects did not have prior exposure to benzodiazepines during the previous year. Furthermore, a number of studies have assessed performance while plasma benzodiazepine concentrations are declining. As Laurijssens and Greenblatt (27) point out, this procedure may mask the development of acute tolerance. In the current study, plasma levels of midazolam remained fairly stable, rising only slightly throughout the infusion period. Another factor that may contribute to reported failures to observe tolerance to midazolam may be that tolerance develops at different rates for different benzodiazepine effects. For example, Tang and colleagues (45) observed more rapid and complete tolerance to the sedative effects than to the motor effects of midazolam in rats. Likewise, Curran (9) found that a population of chronic benzodiazepine users was more tolerant to the sedative than to the amnestic effects of lorazepam and diazepam. Many of the human studies that have failed to find tolerance to midazolam used sleep as the dependent measure [e.g., (3,32)]. In the present study, we examined benzodiazepine-induced disruption of motor control, to which animals have previously been shown to become tolerant (45). Acute tolerance to benzodiazepine psychomotor effects (e.g., digit-symbol substitution) has also been readily demonstrated with a number of different benzodiazepines in humans (11,13,26). We chose to focus on fine motor control to have the opportunity to detect rebound increases in muscle force, which may not have been easily measured using standard psychomotor tasks.

Tang and colleagues (45) trained rats on a motor control task analogous to the visual tracking task used in this study.

One group of rats was given midazolam prior to each motor task session. Over a number of sessions, the rats demonstrated tolerance to midazolam's motor control effects. Tang and colleagues (45) observed some disruption in responding (withdrawal effects) when midazolam administration was terminated after 4 months. (They did not report directional changes in muscle force, so it is not possible to determine if they observed rebound.) However, as in the current study, Tang and colleagues found no evidence for precipitated withdrawal when flumazenil was administered to tolerant rats. They suggest that flumazenil is better at precipitating withdrawal in primates than in rodents, which may account for their failure to observe precipitated withdrawal. Flumazenil does precipitate withdrawal in human subjects (14,40). Therefore, a species difference is not likely to account for our failure to observe rebound.

Flumazenil has been reported to have weak agonist and inverse agonist properties, along with its antagonist effects (12). Such effects may have obscured the observation of rebound. In support of this, Higgitt and colleagues (19) found that flumazenil impairs reaction time and finger tapping in human subjects. Kawasaki and colleagues (22) also found a partial agonist effect of flumazenil on cross extensor reflex in rat quadriceps. However, Bonetti and colleagues (4) found that flumazenil increased muscle tone in rats. Flumazenil did not appear to have intrinsic agonist or inverse agonist effects in the current study; it did not decrease or increase muscle force in subjects, who had previously received saline. Furthermore, the agonist and inverse agonist effects, which have been reported for flumazenil, are typically observed only at high doses. The doses used in this study are below that range. (The lower dose of flumazenil used in the Higgitt study—30 mg—was over 10 times the dose used in this study.) Nevertheless, the flumazenil dosage used here rapidly antagonized the drug effect observed in the visual tracking task, and returned midazolam-infused subjects to baseline levels of performance on this task.

The failure to observe a rebound in muscle force in this study suggests that factors other than increases in muscle force output or control account for tolerance in the subjects receiving midazolam. Tolerance cannot be explained by declining midazolam plasma concentrations, as plasma levels actually increased slightly over the course of the infusion. One possible explanation is that there was a change in the action of midazolam at the benzodiazepine receptor. Ellinwood and colleagues (11), however, found no clear relation between acute tolerance to benzodiazepines and receptor affinity or distribution kinetics of the drugs. Furthermore, Wu and colleagues (49) and Ramsey-Williams and colleagues (37) failed to find any changes in brain benzodiazepine receptor binding in rats chronically treated with midazolam for 3 weeks, even though tolerance to the anticonvulsant effects of midazolam was evident.

It is likely that factors not directly related to changes in muscle force contributed significantly to our findings. Subjects may have improved performance throughout the midazolam infusion because they overcame attentional or sedative effects of midazolam. Benzodiazepines are known to affect the speed of cognitive processing independent of their motor effects (46). Formal measures of sedation were not obtained in this study. However, notes made by experimenters indicate that subjects frequently reported feeling drowsy at the onset of the infusion (often dozing between force assessments), but then later reported feeling more awake as the session continued. Acute tolerance to the sedative and attentional effects of benzodiazepines has been noted by other investigators. Using performance on digit symbol substitution and hand–eye coordination tasks as dependent measures, Kroboth and colleagues (26) observed tolerance to triazolam during 8-h intravenous infusions. They report that Nurse Rated Sedation Scores paralleled their psychomotor task data. Likewise, Smith and Kroboth (43) report acute and chronic tolerance in both sedation and performance deficits on the digit symbol substitution task during hourly oral dosing with alprazolam. Decreases in attention (or increases in sedation) may have impaired performance on the visual tracking task by affecting hand–eye coordination. If the improvements seen here result from recovery of alertness or attention, then one might not expect to observe rebound in the visual tracking task.

Several factors may be improved in future research investigating the development of acute tolerance to midazolam. First, additional training/practice sessions on the motor control tasks should reduce the variability that occurs during the experimental test session. Second, a greater degree of acute tolerance may develop over a longer midazolam administration. For example, subjects receiving midazolam did not become completely tolerant to midazolam's effects on the visual tracking task. Performance at 65 min postinfusion remained worse in subjects receiving midazolam than in subjects receiving saline. Third, by extending the midazolam administration, longer rest periods can be placed between the muscle force assessments to lessen the onset of muscle fatigue. The steady decline in maximum muscle force over the infusion period (particularly evident in the placebo condition) indicates that fatigue did add to the variability of these data. Finally, improvements could be made in the infusion paradigm to achieve even more stable plasma midazolam concentrations. Increasing midazolam concentrations likely contributed to the slight worsening of performance towards the end of the midazolam infusion period. With the incorporation of these improvements, this paradigm shows promise for studying the processes underlying the development of acute tolerance.

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