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Acute Effects of Benzodiazepines on Operant Behavior and In Vivo Receptor Binding in Mice

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BURKE, T. F., L. G. MILLER AND J. M. MOERSCHBAECHER. *Acute effects of benzodiazepines on operant behavior and in vivo receptor binding in mice.* PHARMACOL BIOCHEM BEHAV **48**(1) 69–76, 1994.—Lorazepam and alprazolam produced dose-dependent decreases in the rate of fixed-ratio (FR) 20 schedules of food presentation in which either a nose-poke or a lever-press defined the operant and under a fixed-interval (FI) 2-min lever-press schedule of food presentation. In contrast, under FI 2-min and differential reinforcement of low response rate (DRL) 20-s schedules of nose-poke responding for food, intermediate doses of alprazolam produced increases in response rate. Lorazepam, however, only decreased overall response rates under the FI schedule and produced some increases in responding under the DRL schedule. Acute in vivo benzodiazepine receptor binding experiments showed that low to intermediate doses of alprazolam produced significant increases in the binding of [^{3}H]flumazenil in all brain areas tested, while lorazepam produced increases in the brain stem only. The acute effects on binding produced by both drugs were positively and significantly correlated with their acute effects on response rate only under the FR lever-press procedure. These results indicate that the effects of benzodiazepines on in vivo binding may be related to their effects on FR lever-press responding.

Alprazolam Benzodiazepines Differential reinforcement of low response rate Fixed-interval Fixed-ratio Flumazenil $GABA_A$-benzodiazepine receptor complex In vivo binding Lorazepam Mice Operant behavior

THE benzodiazepines represent one of the most frequently prescribed drug classes in the world. This widespread use is due in large part to their broad range of properties, which include anxiolytic, anticonvulsant, sedative, and muscle relaxant effects (15). These effects are presumed to be mediated primarily by a specific central benzodiazepine receptor which is associated in a macromolecular complex with the recognition site for γ-aminobutyric acid (GABA) and a chloride ion channel.

Benzodiazepines also have well-characterized effects on schedule-controlled behavior. In rats, it has been found that benzodiazepines produce dose-dependent decreases in response rates under schedules of reinforcement controlling high rates of responding, such as fixed-ratio (FR) schedules (27). However, under schedules that engender relatively low response rates, such as fixed-interval (FI) or differential reinforcement of low response rate (DRL) schedules, benzodiazepines often produce either no change or increases in response rate at lower doses and then decrease response rates at higher doses (2,27). These results indicate that benzodiazepines exert rate-dependent effects upon schedule-controlled behavior in a manner similar to those previously described for stimulants such as amphetamine (6). One goal of the present study was to begin to extend the characterization of the effects of benzodiazepines on schedule-controlled behavior to the mouse.

A number of studies have attempted to correlate the behav-

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ioral effects of benzodiazepines with their effects at the receptor level. Generally, in vivo binding techniques, in which receptors are labelled in the brains of intact animals following the systemic administration of a specific radioligand, have been used for this purpose. Ideally, the radioligand used for such techniques should yield high specific to nonspecific binding ratios without requiring homogenization or washing of the tissue, procedures which can greatly affect in vivo receptor binding, in order to reduce nonspecific binding (14). The advantage of this approach is that both the behavioral effects of benzodiazepines and their receptor binding properties can be measured and, therefore, compared under physiological conditions. For example, an early study demonstrated a good correlation between the anticonvulsant activity of several benzodiazepines and the ability of these drugs to inhibit the binding of [^{3}H]flunitrazepam, administered in vivo, in various brain regions of the mouse (8). It was subsequently shown that [^{3}H]flumazenil ([^{3}H]Ro 15-1788) yielded substantially higher specific to nonspecific ratios of in vivo binding in mice than did [^{3}H]flunitrazepam (14). Using this radioligand, correlations have been found between the acute (17) and chronic (23) effects of benzodiazepines on spontaneous motor activity in mice and their effects on the in vivo binding of [^{3}H]flumazenil. A recent study (11), in which benzodiazepine agonists of varying intrinsic efficacies were compared, showed strong relationships between the ability of these drugs to produce their characteristic effects (anticonvulsant, anxiolytic, sedative, and muscle relaxant) and their fractional benzodiazepine receptor occupancy as determined by their displacement of [^{3}H]flumazenil, administered in vivo, from the mouse brain.

The other primary goal of the present study was to determine the extent to which the acute effects of benzodiazepines on operant behavior were related to their acute effects on the in vivo binding of [^{3}H]flumazenil in mice. Lorazepam and alprazolam were chosen for investigation primarily because of differences in their pharmacological and chemical profiles. Lorazepam is structurally and pharmacologically similar to the classical benzodiazepines such as chlordiazepoxide and diazepam (13). Alprazolam, on the other hand, is a member of the triazolobenzodiazepine class and is reported to have a unique spectrum of clinical effects. Like lorazepam, it is commonly prescribed as an anxiolytic agent. However, alprazolam has also been reputed to possess antidepressant (9,12) and antipanic (3) activities in clinical trials. Furthermore, in monkeys, alprazolam and other triazolo-substituted compounds are more disruptive than classical benzodiazepines on acquisition, performance, and memory tasks (1,5,24). Both drugs were investigated under a variety of operant schedules as well as in the in vivo binding procedure outlined above (14).

METHODS

Subjects

Approximately 130 male CD-1 mice (Charles River Laboratories, Wilmington, DE) were used as subjects. Animals used in behavioral procedures were maintained by food presented during the experimental sessions and by postsession supplemental feeding (i.e., food-deprived) and were generally individually housed. All others were fed ad lib and generally group-housed (5–7 mice/cage). Water was continuously available in the home cages. All mice were housed in a temperature-controlled environment under a 12-h light–dark cycle.

Apparatus

Three experimental chambers were used, each consisting of a polypropylene box (with wire lid) measuring 26 × 15 × 20 cm. In one wall of each chamber was an opening measuring 4 × 3 cm leading to a blind corridor approximately 4 cm long. Two chambers were used for a nose-poke procedure. In each of these chambers there was a photobeam cell and detector (Coulbourn Instruments, Lehigh Valley, PA, model nos. T22-01 and S23-01) on either side of the corridor, located 2 cm down its length. Mice were trained to interrupt this photobeam using a nose-poke response. A response was defined as one interruption of the photobeam. The other chamber was used for a lever-press procedure. In this chamber an ultrasensitive lever (Coulbourn Instruments, model no. E22-01) was located approximately 6 cm to the right of the corridor and 1 cm above the floor. A force of less than 0.02 N was required to record a response. In each chamber there was a small hole in the floor of the corridor located 2 cm down its length through which the reinforcer (0.1 ml sweetened condensed milk) was delivered via a dipper (Coulbourn Instruments, model no. E14-05). Each chamber was enclosed in a ventilated, sound-attenuating shell equipped with houselights. The experimental procedures were programmed through the use of solid-state circuitry and the data were recorded on counters, running-time meters, and cumulative recorders.

Behavioral Procedures

Seventeen mice were maintained under an FR 30 schedule of food presentation. Under this schedule, each 30th response resulted in a 2-s presentation of the dipper. The lever-press response defined the operant for 7 mice and the nose-poke defined the operant for the remaining 10 mice.

Nineteen mice were maintained under an FI 2-min schedule. Under this schedule, the first response after each 2-min interval of time had elapsed produced the reinforcer. The lever-press defined the operant for 9 mice and the nose-poke response defined the operant for 10 mice.

Finally, eight mice were maintained under a DRL 20-s schedule. Under this schedule, a response was reinforced only if it occurred at least 20 s following the previous response or reinforcer. The nose-poke response defined the operant under this schedule. Under all schedules each session was 30 min in duration.

Binding Procedure

The remaining 86 mice were used to study the acute effects of lorazepam and alprazolam on the binding of [^{3}H]flumazenil in selected brain regions using a modification of the well-established in vivo binding method of Goeders and Kuhar (14). In the present study, the radioligand was administered by the IP route rather than the IV route which had been employed in other studies. The results of pilot studies revealed that both routes of administration produced similar levels of [^{3}H]flumazenil in the various brain regions over a wide range of time points (Moerschbaecher and Minor, personal communication). The specific binding of [^{3}H]flumazenil was defined as the difference between total binding (vehicle pretreatment) in a particular brain region and the amount of binding that was observed following pretreatment with 10 mg/kg of temazepam. This was experimentally determined to be the lowest dose at which the receptors were saturated (i.e., higher doses produced no further decrease in binding) and, as such, reflected nonspecific binding. This amount was consistently less than 10% of total binding. Vehicle (total binding) or temazepam (nonspecific binding) was administered 30 min prior to [^{3}H]flumazenil, 3 μCi in a volume of 0.15 ml saline. Twenty minutes later the animals were decapitated and their brains

were rapidly removed. The cerebral cortex, cerebellum, hypothalamus, and brain stem were dissected, weighed, and transferred to plastic scintillation vials. The tissues were solubilized by the addition of 2 ml of Protosol and incubation at 40°C for 24 h. Ten milliliters of scintillation fluid was then added and the samples allowed to stand for 24 h at room temperature. Radioactivity was measured by scintillation spectrometry. It should be noted that temazepam is extensively converted to an active metabolite, oxazepam, in mice (29). However, the results obtained using temazepam as the saturating ligand were both qualitatively and quantitatively similar to those obtained when lorazepam, which has no active metabolites (26), was used to measure nonspecific binding. When the effects of the acute administration of lorazepam and alprazolam were determined, the mice were pretreated with varying doses (0.01, 0.032, 0.1, 0.18, 0.32, 0.56, 1, 1.8, and 3.2 mg/kg) of these drugs 5 min prior to the administration of [^{3}H]flumazenil. This pretreatment time was the same as that used in all behavioral experiments. Processing of tissue occurred 20 min after this as outlined above.

Drug Testing

The acute effects of lorazepam and alprazolam were determined in mice responding under all schedules of reinforcement. Additionally, the acute effects of each drug on in vivo binding of [^{3}H]flumazenil were determined as described above. Both drugs were initially dissolved in propylene glycol and subsequently diluted with saline. The drugs were administered by the IP route in a volume of 5 ml/kg body weight. However, when high doses were administered, the injection volume was 10 ml/kg. Drugs were administered 5 min before the start of a behavioral session. Drug sessions for the acute behavioral studies were generally conducted on Tuesdays and Fridays with control sessions (saline) on Thursdays. Doses of each drug were administered in a mixed order. Furthermore, individual doses were repeatedly tested with no consistent effect (i.e., tolerance, amplification of rate increasing effects, etc.) apparent upon these subsequent administrations.

Materials

Lorazepam was obtained from Wyeth Laboratories (Philadelphia), and alprazolam was obtained from The Upjohn Co. (Kalamazoo, MI). [^{3}H]Flumazenil (specific activity 73–78 Ci/mmol) and Protosol were obtained from New England Nuclear (Boston). Scintillation fluid was obtained from Beckman (Fullerton, CA). All other materials and reagents were obtained from standard commercial sources.

Data Analysis

The data for each operant procedure were analyzed in terms of the overall response rate, which was expressed as a percentage of the control response rate. The percentage of control response rate for each drug dose was calculated based on the saline control response rate immediately preceding the administration of that dose of drug. The data for all subjects receiving a particular drug under a particular procedure were then averaged to yield a dose–effect curve. These data were then subjected to a one-way analysis of variance (ANOVA). If a significant difference was found in the overall ANOVA, further analysis was undertaken using a one-sample least significant difference test, based on the pooled variance estimate obtained from the ANOVA (28), by comparing the mean at each dose of drug to control (100%). For the acute receptor binding assays, the data were analyzed in terms of specific binding, which was expressed as percent of control (vehicle). These data were also analyzed in an identical fashion as described above. Pearson's correlation coefficient was used to evaluate the relationship between specific binding and response rate, and Student's t test was used to test for the significance of each correlation (Systat, Evanston, IL; Version 3.0).

RESULTS

No consistent effects of saline (control) injections were observed in any procedure. The FR 20 schedules engendered the highest control rates of responding. Additionally, those rates obtained using the nose-poke operant (mean for all eight subjects of 3.22 responses/s ± SEM of 0.17) were substantially higher than those obtained using the lever-press operant (1.63 ± 0.17; n = 6). Similarly, the nose-poke operant produced higher control rates of responding (0.31 ± 0.05; n = 6) under the FI 2-min schedule than did the lever-press (0.18 ± 0.02; n = 9), although overall rates were much lower under the FI schedule than they were under the FR schedule for both operants. Finally, the DRL nose-poke schedule produced the lowest control rates of responding of all the schedules used in the present study (0.10 ± 0.01; n = 8).

A comparison of the effects of lorazepam on FR nose-poke and lever-press responding is shown in the top panel of Fig. 1. Lorazepam decreased the overall rates of both operants in a dose-dependent manner. However, the lever-press procedure

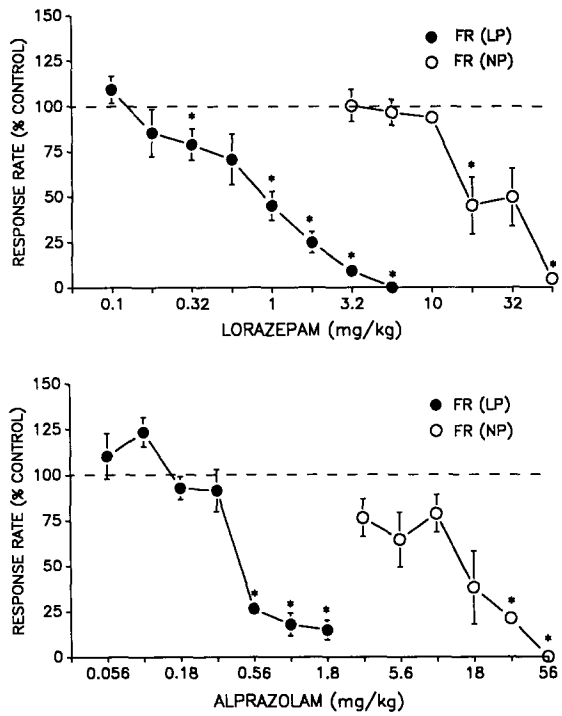

FIG. 1. Effects of varying acute doses of lorazepam and alprazolam on overall response rate (expressed as percentage of saline control as described in Methods) under both the fixed-ratio (FR) 30 lever-press and FR 30 nose-poke operants. The points and vertical lines represent the means and standard errors of all mice tested at each particular dose. The points without vertical lines indicate either a single determination or an instance in which the standard errors are encompassed by the point. *p < .05 compared to control (dashed line).

was 10 times more sensitive to the rate-decreasing effects of lorazepam than was the nose-poke procedure. Similar results obtained with alprazolam, as shown in the bottom panel of Fig. 1. Alprazolam also produced only dose-dependent decreases in response rates, with the lever-press approximately 10 times more sensitive to these effects than the nose-poke response. Interestingly, both drugs exhibited nearly equal potencies under each operant in terms of their rate-decreasing effects.

The acute effects of varying doses of lorazepam on the overall rate of FI 2-min nose-poke and lever-press responding are shown in the top panel of Fig. 2. Lorazepam generally produced dose-dependent decreases in overall response rates under both schedules. In contrast, a significant increase in response rate was produced by alprazolam under the FI nose-poke schedule at a dose of 0.1 mg/kg (Fig. 2, bottom panel). While in some animals other doses produced substantial increases in rate, these increases were not statistically significant due to the large amount of variability among subjects. However, alprazolam, like lorazepam, produced only dose-dependent decreases in response rate under the FI lever-press schedule. Lower doses of each drug were required to decrease rate under the lever-press operant.

Figure 3 shows the effects of lorazepam and alprazolam on response rate under the DRL nose-poke schedule. Lorazepam produced significant increases in response rate across a range

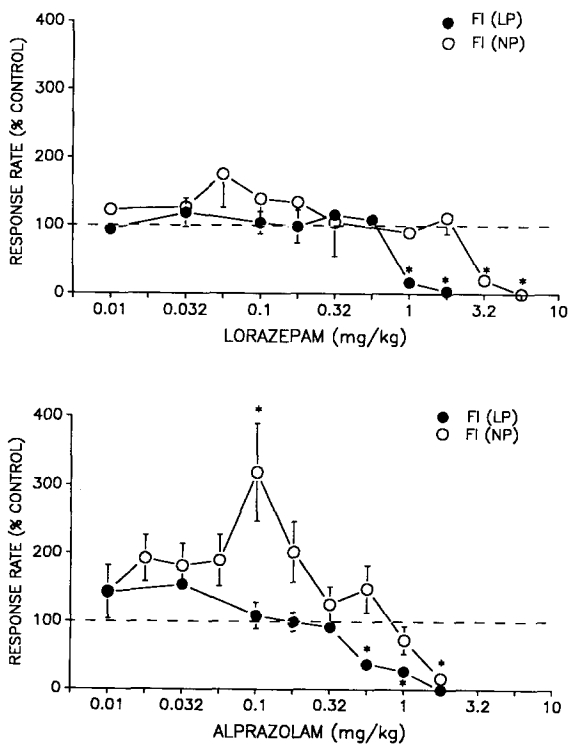

FIG. 2. Effects of varying acute doses of both lorazepam and alprazolam on overall response rate under both the fixed-interval (FI) 2-min lever-press and FI 2-min nose-poke operants. The points and vertical lines represent the means and standard errors of all mice tested at each particular dose. The points without vertical lines indicate either a single determination or an instance in which the standard errors are encompassed by the point. *$p < .05$ compared to control (dashed line).

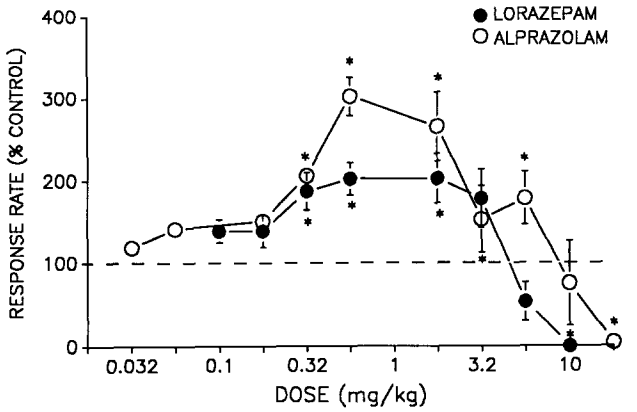

FIG. 3. Effects of varying acute doses of lorazepam and alprazolam on overall response rate under the DRL 20-s nose-poke schedule. The points and vertical lines represent the means and standard errors of all mice tested at each particular dose. The points without vertical lines indicate either a single determination or an instance in which the standard errors are encompassed by the point. *$p < .05$ compared to control (dashed line).

of doses (0.32–3.2 mg/kg). This schedule was slightly less sensitive to the rate-decreasing effects of lorazepam than was the FI nose-poke schedule. Rate-increasing effects were also produced by alprazolam under the DRL schedule across a dose range of 0.32–5.6 mg/kg (except at 3.2 mg/kg). The increases in response rates produced by certain doses of alprazolam were of equal magnitude to those obtained under the FI schedule. However, unlike the results obtained with lorazepam, the DRL schedule was approximately 10 times less sensitive to the rate-decreasing effects of alprazolam than was the FI schedule.

In addition to the acute effects of the two drugs on several schedules of reinforcement, their acute effects on receptor binding were also determined in the cerebellum, brain stem, cortex, and hypothalamus. Acute administration of [^{3}H]flumazenil revealed that the level of binding was highest in the hypothalamus (41.39 ± 5.00 cpm/mg wet tissue weight) and cortex (34.65 ± 2.64 cpm/mg), intermediate in the cerebellum (18.83 ± 1.55 cpm/mg), and lowest in the brain stem (8.5 ± 0.5 cpm/mg). Figure 4 compares the acute effects of lorazepam and alprazolam on the binding of [^{3}H]flumazenil in each of these brain regions. Acute lorazepam administration produced only decreases in binding in the cerebellum, while it produced significant increases in the brain stem at doses of 0.1 and 0.18 mg/kg. However, the dose-effect function for alprazolam was an inverted-U shape in both the cerebellum and brain stem. That is, low doses had no effect, intermediate doses produced significant increases in binding, and higher doses decreased binding in a dose-dependent manner. This relationship was obtained with alprazolam in all brain regions studied in the acute administration experiments. In both the cerebellum and brain stem, alprazolam produced increases at doses of 0.1 and 0.18 mg/kg, with the peak effect occurring at 0.1 mg/kg. The results in the cortex and the hypothalamus were similar to those seen in the cerebellum in that lorazepam produced only dose-dependent decreases in binding and alprazolam produced significant increases at 0.1 mg/kg. However, the increases in in vivo binding produced by alprazolam were of a smaller magnitude in the cortex and hypothalamus.

Table 1 shows the results of a correlational analysis per-

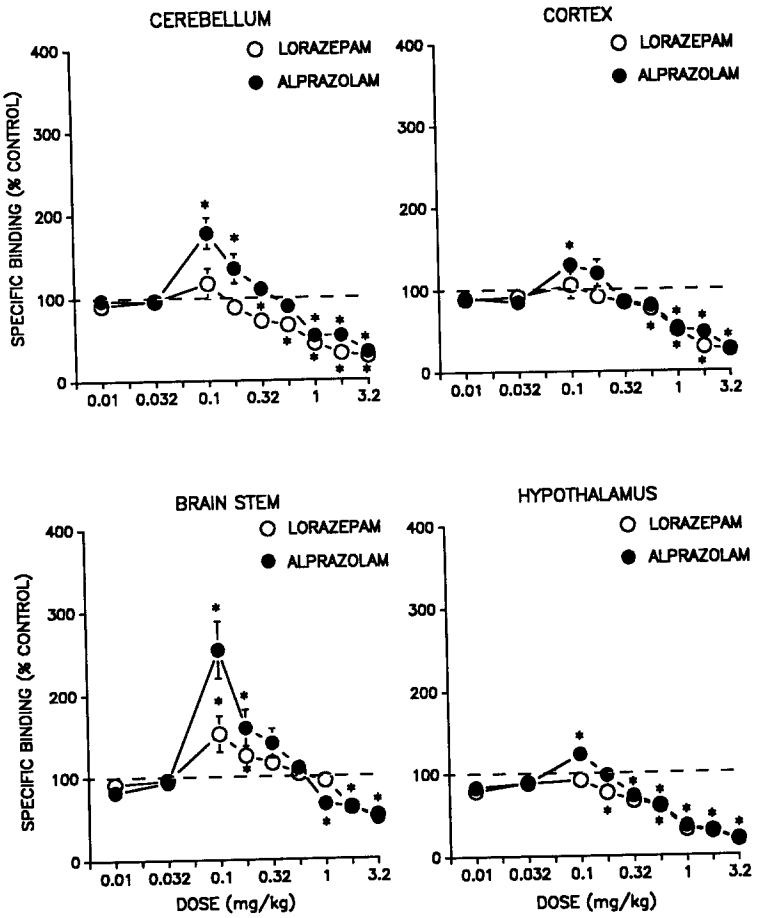

FIG. 4. Effects of varying acute doses of lorazepam and alprazolam on in vivo receptor binding in the cerebellum, cortex, brain stem, and hypothalamus. The points and vertical lines represent the means and standard errors of all mice tested at a particular dose. The points without vertical lines indicate either a single determination or an instance in which the standard errors are encompassed by the point. *$p < .05$ compared to control (dashed line).

formed between the acute effects of alprazolam and lorazepam on response rates under all schedules of reinforcement (except the FR nose-poke) and their acute effects on the in vivo binding of [^{3}H]flumazenil in each brain region. These correlations were performed using the values of each dependent variable across the range of doses used in the binding study: 0.01–3.2 mg/kg of each drug. The response rate data from the FR nose-poke procedure could not be analyzed in

TABLE 1

CORRELATION COEFFICIENTS

	FR-LP		FI-NP		FI-LP		DRL-NP	
	ALPZ	LZPM	ALPZ	LZPM	ALPZ	LZPM	ALPZ	LPZM
CB	0.95*	0.97*	0.94*	0.77	0.57	0.80	−0.17	−0.73
CX	0.91*	0.99*	0.94*	0.77	0.65	0.91*	−0.16	−0.61
HY	0.94*	0.98*	0.96*	0.80	0.79	0.88*	−0.36	−0.64
BS	0.93*	0.99*	0.88*	0.70	0.34	0.61	−0.07	−0.66

Correlation coefficients (r values) for the relationship between the effects of alprazolam (ALPZ; 0.01–3.2 mg/kg) and lorazepam (LZPM; 0.01–3.2 mg/kg) on response rates under the FR lever-press (FR-LP), FI nose-poke (FI-NP), FI lever-press (FI-LP), and DRL nose-poke (DRL-NP) schedules of reinforcement and their effects on in vivo binding of [^{3}H]flumazenil in the cerebellum (CB), cortex (CX), hypothalamus (HY), and brain stem (BS). *$p < 0.05$.

this manner as the effective dose ranges for each drug in decreasing response rates were outside those for each drug in producing decreases in the in vivo binding of [^{3}H]flumazenil. The acute effects of both drugs on rate under the FR lever-press schedule were found to be positively and significantly correlated with their acute effects on in vivo receptor binding in all brain areas tested. Furthermore, the effects of alprazolam on response rate under the FI nose-poke schedule were positively and significantly correlated with its effects on binding in all brain regions, while the effects of lorazepam were not. Other significant correlations were obtained between the effects of lorazepam on response rate under the FI lever-press schedule and its effects on in vivo binding in the cortex and hypothalamus. Interestingly, the effects of both drugs on response rate under the DRL nose-poke schedule generated negative but nonsignificant correlation coefficients in all brain areas tested.

DISCUSSION

Some of the effects of lorazepam and alprazolam on response rate were consistent with previous literature concerning the rate-dependent effects of benzodiazepines in other species. For instance, both drugs produced only decreases in response rate under both FR procedures, which engendered the highest control rates of responding. Intermediate doses of each drug also increased response rates under the DRL nose-poke schedule, which engendered the lowest control response rates. In addition, one dose of alprazolam (0.1 mg/kg) significantly increased response rate under another schedule with relatively low control response rates, the FI nose-poke.

On the other hand, neither drug produced any increase in response rate under the FI lever-press procedure, a schedule which produced control rates of responding similar to the DRL nose-poke schedule, and lorazepam produced only slight but nonsignificant increases in rate under the FI nose-poke procedure. Consistent with these findings, it has been reported that after administration of benzodiazepines increases in responding are usually greater in DRL than in FI schedules (4). Nevertheless, if the effects of lorazepam and alprazolam on response rates under the FR and DRL schedules were due solely to rate dependence, it might be expected that consistent increases in responding would occur at intermediate doses of these drugs under both FI procedures (6). However, this pattern of effects was not apparent in the present study.

Interestingly, the FR nose-poke procedure was considerably less sensitive to rate-decreasing effects than was the FR lever-press procedure. The reason for this difference between the FR operants is not readily clear. Under the conditions of the present study, the nose-poke response appeared to be of an inherently less discrete nature than the lever-press response. For example, it was observed that after the administration of high doses of both drugs mice were capable of responding under the FR nose-poke schedule, even though visibly intoxicated, whereas such doses would eliminate responding under the FR lever-press schedule. Also, unlike the lever-press procedure, consummatory behavior and nose-poke responding occurred in the same place, and this may also account for some of the differential sensitivity between the two schedules, although its exact role is unclear. In addition, the rate-decreasing effects under the FR nose-poke schedule occurred at doses well beyond those which were required to produce full displacement of [^{3}H]flumazenil binding. This may suggest that the rate-decreasing properties of these drugs under the FR nose-poke procedure represented a combination of both benzodiazepine receptor- and non-benzodiazepine receptor-mediated effects.

In contrast, the rate-decreasing effects of these same drugs under the FR lever-press schedule occurred across a range of doses at which gross motor deficits were not readily apparent. Interestingly, these rate-decreasing effects occurred across a similar dose range as those that were found to produce decreases in in vivo receptor binding. In fact, the effects of both drugs on response rate under this schedule were positively and significantly correlated with their effects on the in vivo binding of [^{3}H]flumazenil in all brain regions studied. These findings suggest that the rate-decreasing effects of the two drugs under the FR lever-press procedure represent benzodiazepine receptor-mediated effects.

In general, the effects of lorazepam and alprazolam on response rates under the schedules which produced low control rates of responding were not consistently correlated with their effects on the in vivo binding of [^{3}H]flumazenil in all but one instance (i.e., the effects of alprazolam under the FI nose-poke procedure). Although the high correlation observed between the effects of alprazolam on FI response rate and its effects on the in vivo binding of [^{3}H]flumazenil is intriguing, the FI nose-poke schedule yielded results which are probably too inconsistent to be considered a reliable indicator of benzodiazepine receptor-mediated effects. Furthermore, it would seem unlikely that an increase in benzodiazepine receptor binding would result in an increase in response rate. Indeed, it might be anticipated that an increase in benzodiazepine binding would enhance the depressant actions of alprazolam via GABA and be reflected instead as a decrease in response rate. The effects of lorazepam and alprazolam on response rates under the DRL nose-poke schedule do not appear to be strictly benzodiazepine receptor-mediated effects either. If anything, the effects of both drugs on in vivo binding tended toward negative correlations with their effects on response rate under the DRL schedule.

The regional distribution of benzodiazepine receptors, as determined by acute administration of [^{3}H]flumazenil (cortex and hypothalamus > cerebellum > brain stem), was consistent with previously reported results (21). Lorazepam, at low doses, produced some slight increases in binding, but these increases were statistically significant only in the brain stem. In contrast, low doses of alprazolam produced significant increases in the specific in vivo binding of [^{3}H]flumazenil in all brain regions. The greatest increases in acute in vivo binding observed with both drugs occurred in the brain stem, which exhibited the lowest control levels of binding, and the smallest increases occurred in the hypothalamus, which exhibited the highest control levels of binding. These results are in basic agreement with a number of previous reports which illustrated the relative inability of lorazepam to produce increases in binding (22) as well as the ability of low doses of alprazolam to produce significant increases in the specific in vivo binding of [^{3}H]flumazenil (16,17,20).

The decreases in in vivo [^{3}H]flumazenil binding following high doses of alprazolam can be explained by the fact that high levels of alprazolam at the receptor can displace the radioligand in a dose-dependent manner. However, it is much more difficult to understand why binding significantly increases following the acute administration of low doses of alprazolam. This effect does not appear to be a general characteristic of triazolo-substituted benzodiazepines, as it has been reported that triazolam, another triazolobenzodiazepine, does not produce increases in in vivo benzodiazepine receptor binding (20). One possible explanation for the ability of alpra-

zolam to enhance in vivo binding may be its interaction with central adrenergic systems. It has been reported that alprazolam is capable of stimulating α_2 adrenoceptors (10). Certain GABAergic nerve terminals in the rat brain have been shown to possess α_2 receptors which, when stimulated, enhance GABA release (25). This increased GABAergic activity might then result in enhanced benzodiazepine binding via allosteric modifications of the $GABA_A$-benzodiazepine receptor complex (18,31,32). Another possibility involves the existence of multiple subtypes of benzodiazepine receptors. Recently, at least two subtypes which are differentiated by the particular isoform of the alpha subunit of the $GABA_A$ receptor with which they are complexed have been characterized (7). Current in vitro evidence suggests that neither alprazolam nor Ro 15-1788 is capable of distinguishing between type 1 and type 2 central benzodiazepine receptors (19). However, it is believed that even more pharmacologically distinct $GABA_A$-benzodiazepine receptor subtypes exist, although they have not yet been identified. This postulated heterogeneity is based on the large number of different possible combinations of the various $GABA_A$ receptor subunits (alpha, beta, and gamma) as well as on the mounting evidence for $GABA_A$ receptors with multiple alpha subunits (7,30). Furthermore, it is likely that these different subtypes will exhibit different binding properties for various benzodiazepines. These subtypes may also be capable of interacting with and modulating each other. Perhaps it is through such a mechanism that alprazolam can trigger the event that allows [^{3}H]flumazenil to label an increased number of benzodiazepine receptors. Undoubtedly, the issue of $GABA_A$-benzodiazepine receptor subtypes must be resolved in much greater detail before such potential interactions can be fully investigated.

In summary, the present study extended the characterization of the effects of benzodiazepines on schedule-controlled behavior to the mouse and was consistent with previous studies concerning the effects of benzodiazepines on the in vivo binding of [^{3}H]flumazenil. Lorazepam and alprazolam produced effects on responding under both FR procedures and the DRL schedule that appear to be rate-dependent in nature. However, the effects of these drugs on responding under both FI procedures cannot be generally classified as rate-dependent. In addition, the two drugs produced dissimilar effects on the in vivo binding of [^{3}H]flumazenil. Low doses of alprazolam produced increases in binding in all brain regions studied, whereas low doses of lorazepam did not (except in the brain stem). Finally, the effects of lorazepam and alprazolam under the FR lever-press procedure were significantly and positively correlated with their effects on the in vivo binding of [^{3}H]flumazenil. Perhaps this finding indicates that the effects of benzodiazepines on in vivo binding may be reflected in their effects on FR lever-press responding. Although further study is needed to establish the generality of this finding, this procedure may prove to be useful in establishing relationships between the acute and chronic neurochemical effects of benzodiazepines and their behavioral consequences.

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REFERENCES

1. Brocklehurst, C.; Devia, C.; Faust, B.; Moerschbaecher, J. M. A comparison of the effects of triazolam and diazepam on the acquisition of conditional discriminations in monkeys. Fed. Proc. 46:1131; 1987.
2. Canon, J. G.; Lippa, A. S. Use of DRL in differentiating anxiolytic and neuroleptic properties of CNS drugs. Pharmacol. Biochem. Behav. 6:591–593; 1977.
3. Ciraulo, C. A.; Barnhill, J. G.; Boxenbaum, H. G.; Greenblatt, D. J.; Smith, R. B. Pharmacokinetics and clinical effects of alprazolam following single and multiple oral doses in patients with panic disorder. J. Clin. Pharmacol. 4:292–298; 1986.
4. Dantzer, R. Behavioral effects of benzodiazepines: A review. Biobehav. Rev. 1:71–86; 1977.
5. Devia, C.; Brocklehurst, C.; Faust, B.; Moerschbaecher, J. M. A comparison of the effects of triazolam, diazepam, and oxazepam on a fixed-ratio discrimination in monkeys. Fed. Proc. 46:1131; 1987.
6. Dews, P. B.; Wenger, G. R. Rate-dependency of the behavioral effects of amphetamine. In: Dews, P. B.; Thompson, T., eds. Advances in behavioral pharmacology. New York: Academic Press; 1977:167–227.
7. Doble, A.; Martin, I. L. Multiple benzodiazepine receptors: No reason for anxiety. Trends Pharmacol. Sci. 13:76–81; 1992.
8. Duka, T.; Hollt, V.; Herz, A. In vivo receptor occupancy by benzodiazepines and correlation with the pharmacological effect. Brain Res. 179:147–156; 1979.
9. Eriksson, B.; Nagy, A.; Starmark, J. E.; Thelander, U. Alprazolam compared to amitriptyline in the treatment of major depression. Acta Psychiatr. Scand. 75:656–663; 1987.
10. Eriksson, E.; Carlsson, M.; Nilsson, C.; Soderpalm, B. Does alprazolam, in contrast to diazepam, activate alpha-2 adrenoceptors involved in the regulation of rat growth hormone secretion? Life Sci. 38:1491–1498; 1986.
11. Facklam, M.; Schoch, P.; Bonetti, E. P.; Jenck, F.; Martin, J. R.; Moreau, J.-L.; Haefely, W. Relationship between benzodiazepine receptor occupancy and functional effects in vivo of four ligands of differing intrinsic efficacies. J. Pharmacol. Exp. Ther. 261:1113–1121; 1992.
12. Feighner, J. P.; Aden, G. C.; Favre, L. F.; Rickles, K.; Smith, W. T. Comparison of alprazolam, imipramine, and placebo in the treatment of depression. JAMA 249:3057–3064; 1983.
13. Gluckman, M. I.; Stein, L. Pharmacology of lorazepam. J. Clin. Psychiatry 39:3–10; 1978.
14. Goeders, N. E.; Kuhar, M. J. Benzodiazepine receptor binding in vivo with [^{3}H] Ro 15-1788. Life Sci. 37:345–355; 1985.
15. Harvey, S. C. Hypnotics and sedatives. In: Gilman, A. G.; Goodman, L. S.; Rall, T. W.; Murad, F. The pharmacological basis of therapeutics, 7th ed. New York: Macmillan; 1985:339–371.
16. Kaplan, G. B.; Tai, N. T.; Greenblatt, D. J.; Shader, R. I. Separate and combined effects of caffeine and alprazolam on motor activity and benzodiazepine receptor binding in vivo. Psychopharmacology 101:539–544; 1990.
17. Lopez, F.; Miller, L. G.; Greenblatt, D. J.; Paul, S. M.; Shader, R. I. Low-dose alprazolam augments motor activity in mice. Pharmacol. Biochem. Behav. 30:511–513; 1988.
18. Marangos, P. J.; Martino, A. M. Studies on the relationship of gamma-aminobutyric acid-stimulated diazepam binding and the gamma-aminobutyric acid receptor. Mol. Pharmacol. 20:16–21; 1981.
19. McCabe, R. T.; Mahan, D. R.; Smith, R. B.; Wamsley, J. K. Characterization of [^{3}H] alprazolam binding to central benzodiazepine receptors. Pharmacol. Biochem. Behav. 37:365–370; 1990.
20. Miller, L. G.; Greenblatt, D. J.; Barnhill, J. G.; Deutsch, S. I.; Shader, R. I.; Paul, S. M. Benzodiazepine receptor binding of triazolobenzodiazepines in vivo: Increased receptor number with low-dose alprazolam. J. Neurochem. 49:1595–1601; 1987.
21. Miller, L. G.; Greenblatt, D. J.; Barnhill, J. G.; Summer, W.

R.; Shader, R. I. "GABA shift" in vivo: Enhancement of benzodiazepine binding in vivo by modulation of endogenous GABA. Eur. J. Pharmacol. 148:123–130; 1988.
22. Miller, L. G.; Greenblatt, D. J.; Paul, S. M.; Shader, R. I. Benzodiazepine receptor occupancy in vivo: Correlation with brain concentrations and pharmacodynamic actions. J. Pharmacol. Exp. Ther. 240:516–522; 1987.
23. Miller, L. G.; Woolverton, S.; Greenblatt, D. J.; Lopez, F.; Roy, R. B.; Shader, R. I. Chronic benzodiazepine administration IV. Rapid development of tolerance and receptor downregulation associated with alprazolam administration. Biochem. Pharmacol. 38:3773–3777; 1989.
24. Moerschbaecher, J. M.; LaMotte, L.; Pierson, M. Effects of alprazolam and temazepam on the retention of conditional discriminations in monkeys. FASEB J. A5568; 1989.
25. Pittaluga, A.; Raiteri, M. GABAergic nerve terminals in rat hippocampus possess α-2 adrenoceptors regulating GABA release. Neurosci. Lett. 76:363–367; 1987.
26. Ruelius, H. W. Comparative metabolism of lorazepam in man and four animal species. J. Clin. Psychiatry 39:11–15; 1978.
27. Sanger, D. J.; Blackman, D. E. Rate-dependence and the effects of the benzodiazepines. In: Thompson, T.; Dews, P. B.; McKim, W. A., eds. Advances in behavioral pharmacology. New York: Academic Press; 1981:1–20.
28. Saville, D. J. Multiple comparison procedures: The practical solution. Am. Statistician 44:174–180; 1990.
29. Schwarz, H. J. Pharmacokinetics and metabolism of temazepam in man and several animal species. Br. J. Clin. Pharmacol. 8: 23S–29S; 1979.
30. Sieghart, W. Multiplicity of $GABA_A$-benzodiazepine receptors. Trends Pharmacol. Sci. 10:407–411; 1989.
31. Tallman, J. F.; Paul, S. M.; Skolnick, P.; Gallagher, D. W. Receptors for the age of anxiety: Pharmacology of the benzodiazepines. Science 207:274–281; 1980.
32. Ticku, M. K. Benzodiazepine-GABA receptor-ionophore complex: Current concepts. Neuropharmacology 22:1459–1470; 1983.