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Chronic benzodiazepine treatment does not alter interactions between positive GABA_A modulators and flumazenil or pentylenetetrazole in monkeys

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Abstract

Benzodiazepines and neuroactive steroids are positive GABA_A modulators acting at distinct binding sites; during benzodiazepine treatment, tolerance develops to many behavioral effects of benzodiazepines, although cross tolerance does not develop to neuroactive steroids. To determine whether differential changes in binding sites contribute to these behavioral differences, interactions between GABA_A modulators were studied in two groups of four monkeys: one group discriminated 0.178 mg/kg of the benzodiazepine midazolam; the other received 5.6 mg/kg/day diazepam and discriminated 0.1 mg/kg flumazenil, which binds to benzodiazepine sites without modulating GABA_A receptors. In untreated monkeys, flumazenil antagonized midazolam but not the neuroactive steroid pregnanolone, while pentylenetetrazole (a negative modulator acting at a third site) antagonized both positive modulators. In diazepam-treated monkeys, 0.1 mg/kg flumazenil or 32 mg/kg pentylenetetrazole produced flumazenil-lever responding, which was reversed by midazolam and pregnanolone. As flumazenil dose increased, larger doses of midazolam, but not pregnanolone, were needed to reverse flumazenil-lever responding. When the pentylenetetrazole dose increased, larger doses of both positive modulators were needed. Thus, interactions between GABA_A modulators were not different between diazepam-treated and untreated monkeys and do not reveal changes in binding sites that could account for differences between benzodiazepines and neuroactive steroids.

Keywords

diazepam; flumazenil; GABA_A modulators; pregnanolone; neuroactive steroids; pentylenetetrazole; monkeys; drug discrimination; monkey

Benzodiazepines are positive modulators of γ -aminobutyric acid_A (GABA_A) receptors, and when they bind to their site on this receptor complex, they facilitate the actions of GABA, thereby increasing Cl⁻ flux. Other binding sites on GABA_A receptors include neuroactive steroid and barbiturate sites, through which drugs can also modulate this receptor complex. Although they act at different binding sites, positive GABA_A modulators produce similar acute behavioral effects. In contrast to their acute effects, several differences have been observed between benzodiazepines and neuroactive steroids during chronic treatment. First, tolerance develops to benzodiazepines, including their effects on seizure activity (Gonsalves and Gallagher 1987; Loscher et al. 1996) and schedule-controlled behavior (McMahon and France 2002b; Gerak 2009), but tolerance does not develop to those effects of neuroactive

steroids (Kokate et al. 1998; Reddy and Rogawski 2000; McMahon and France 2002a). Second, during chronic benzodiazepine treatment, the potency of neuroactive steroids to alter rates of responding does not change, indicating that cross tolerance does not develop to neuroactive steroids (Gerak 2009). Finally, in diazepam-treated monkeys discriminating flumazenil, temporary discontinuation of diazepam treatment results in the emergence of withdrawal signs as well as responding on the flumazenil lever (McMahon et al. 2007), suggesting that monkeys are dependent on diazepam and that flumazenil-lever responding is related to benzodiazepine withdrawal. Regardless of their site of action, positive modulators reverse withdrawal signs, including flumazenil-lever responding; however, relatively smaller doses of neuroactive steroids are needed, as compared to the doses of benzodiazepines (McMahon et al. 2007).

Several effects have been observed in vitro that might contribute to differences between benzodiazepines and neuroactive steroids reported in vivo. For example, chronic benzodiazepine treatment uncouples the benzodiazepine binding site from the GABA_A, barbiturate and neuroactive steroid binding sites (Tietz et al. 1989; Hu and Ticku 1994a; Friedman et al. 1996). Moreover, chronic benzodiazepine treatment decreases mRNA levels for some subunits and increases mRNA levels for others in rat cortex, thereby altering subunit composition and binding sites of GABA_A receptors (Heninger et al. 1990; Kang and Miller 1991; Holt et al. 1996). Such changes in binding sites in vitro might contribute to in vivo differences among these drugs during chronic benzodiazepine treatment.

If binding sites on GABA_A receptors are altered by daily diazepam administration, then interactions between GABA_A modulators might also be expected to change for some drug combinations. In addition to binding site selectivity, GABA_A modulators can differ on many other dimensions, including efficacy. For example, while positive modulators facilitate the actions of GABA, negative modulators inhibit, and a drug like flumazenil has little impact on its actions. In untreated monkeys, interactions have been studied extensively between benzodiazepines and drugs with lower efficacy that act at benzodiazepine sites, including flumazenil and negative modulators; under those conditions, the lower-efficacy drugs antagonize the effects of benzodiazepines (Paronis and Bergman 1999; Lelas et al. 2000). When drugs acting at different sites are studied in combination, negative modulators, but not flumazenil, attenuate the effects of positive modulators (McMahon and France 2006). If binding sites are altered by chronic diazepam administration, then interactions different from those observed in untreated monkeys would be predicted in diazepam-treated monkeys.

The present studies examined drug interactions in two groups of monkeys: one group discriminated the benzodiazepine midazolam and the second group received diazepam daily and discriminated flumazenil. Interactions were examined in both groups by studying flumazenil and pentylenetetrazole, a negative GABA_A modulator acting at a third, distinct binding site (Allan and Harris 1986), in combination with midazolam and pregnanolone. Pentylenetetrazole should attenuate the discriminative stimulus effects of midazolam and pregnanolone whereas flumazenil should attenuate the effects of midazolam and not pregnanolone. To study these interactions in diazepam-treated monkeys, responding on the flumazenil lever was established by administering a single dose of either flumazenil or pentylenetetrazole; thereafter, increasing doses of a positive GABA_A modulator were administered to reverse flumazenil-lever responding. Shifts in dose-effect curves of the positive modulators were compared between the two groups by examining changes in dose-effect curves as the dose of flumazenil or pentylenetetrazole was increased. Changes in GABA_A receptors in untreated and diazepam-treated monkeys might be reflected by quantitative or qualitative differences in interactions between GABA_A modulators.

Methods

Subjects

Five female and three male adult rhesus monkeys weighed between 7.0 and 9.5 kg during these studies. They were housed individually on a 14-h light and 10-h dark cycle with unlimited access to water. One female and three male monkeys received 5.6 mg/kg of diazepam daily and discriminated flumazenil while the other four female monkeys did not receive daily treatment and discriminated midazolam; monkeys had participated in drug discrimination procedures for at least one year before the start of these studies. Free-feeding weights were maintained with primate chow (Harlan Teklad, High Protein Monkey Diet, Madison, WI), fresh fruit and peanuts provided in the home cage; monkeys discriminating flumazenil also received banana-flavored pellets during experimental sessions. Monkeys used in these studies were maintained in accordance with the Institutional Animal Care and Use Committee, The University of Texas Health Science Center at San Antonio, San Antonio, TX, and with the 1996 Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Science, National Research Council, National Academy of Sciences).

Apparatus

Monkeys were placed in chairs (Primate Products, Miami, FL), which were put into ventilated, sound-attenuating chambers during sessions. Each chamber was equipped with two stimulus lights, two response levers, a food receptacle and a food hopper that could deliver 300 mg banana-flavored food pellets (Bio Serv, Inc, Frenchtown, NJ) to the receptacle. The feet of monkeys discriminating midazolam were placed in shoes that were mounted to the front of chairs, and contained brass electrodes to which brief (250 msec, 3mA) electric shock could be delivered from an a.c. shock generator located outside chambers. Computers that controlled experiments and recorded data were connected to chambers by a commercially available interface (Med Associates Inc., East Fairfield, VT, USA).

Procedures

Flumazenil discrimination—Monkeys received 5.6 mg/kg/day of diazepam (p.o.) 3 h before experimental sessions during which they discriminated 0.1 mg/kg of flumazenil while responding under a fixed-ratio 5 schedule of food presentation (McMahon et al. 2007). Experimental sessions lasted up to 2 h and were divided into 15-min cycles. Each cycle began with a 10-min timeout period during which chambers were dark and responding had no programmed consequence. The timeout period was followed by a 5-min response period during which the schedule of food presentation was in effect. Illumination of green lights signaled the beginning of the response period and 5 consecutive responses on the lever designated correct by the injection administered at the beginning of the cycle resulted in the delivery of a food pellet. Following flumazenil administration, the left lever was designated correct for two monkeys and the right lever was designated correct for the other two monkeys. Incorrect responses reset the fixed-ratio requirement on the correct lever. A response period ended after the delivery of 10 food pellets or 5 min, whichever occurred first; any time remaining between the end of a response period and the beginning of the next cycle was a timeout period.

Midazolam discrimination—Monkeys not receiving daily treatment discriminated 0.178 mg/kg of midazolam while responding under a fixed-ratio 10 schedule of stimulus-shock termination (e.g., Gerak et al. 2008). Experimental sessions were also divided into 15-min cycles that began with a 10-min timeout followed by a 5-min response period. Illumination of red stimulus lights signaled the beginning of the response period. During training

sessions, monkeys could extinguish red lights, and postpone the shock schedule for 30 s, by responding 10 consecutive times on the lever designated correct by the injection administered during the first minute of the cycle. Following midazolam administration, the left lever was designated correct for two monkeys, and the right lever was designated correct for the other two monkeys. Incorrect responses reset the fixed-ratio requirement on the correct lever. If monkeys did not satisfy the fixed-ratio requirement within 15 s of illumination of red lights, brief electric shock was delivered. Thereafter, shock was delivered every 15 s until the response requirement was satisfied, the cycle ended, or four shocks were delivered, whichever occurred first.

Training sessions—During training sessions, the training drug, saline or sham injections were administered during the first minute of each cycle. On some occasions, monkeys received saline or sham injections prior to all cycles, with the number of cycles varying between 2 and 8. On other occasions, monkeys received their training dose during one cycle followed by a single sham injection during the next cycle, with responding on the drug-appropriate lever either resulting in the delivery of a food pellet (monkeys discriminating flumazenil) or extinguishing lights and postponing the shock schedule (monkeys discriminating midazolam) for both cycles. The cycle during which the training drug was administered was preceded by 0-6 cycles during which saline or sham injections were administered. Monkeys had previously satisfied more stringent criteria (Lelas et al. 1999; Gerak and France 1999); for the present studies, stimulus control was considered adequate for testing when the following criteria were satisfied during two consecutive training sessions: $\geq 80\%$ responding on the injection-appropriate lever during each cycle and fewer than 5 (monkeys discriminating flumazenil) or 10 (monkeys discriminating midazolam) responses on the incorrect lever prior to completion of the first fixed ratio on the correct lever. When monkeys did not satisfy the testing criteria, training continued until the criteria were satisfied during two consecutive sessions.

Test sessions—Test sessions were identical to training sessions except that satisfying the fixed-ratio requirement on either lever resulted in the delivery of food or postponed shock, and various doses of test compounds were administered either immediately before sessions or during the first minute of each cycle. Initially, the time course of the discriminative stimulus effects of flumazenil and pentylenetetrazole was studied in monkeys discriminating flumazenil by administering a single dose immediately before sessions comprising 6 sham cycles. Thereafter, drug combinations were studied with either flumazenil or pentylenetetrazole administered on the first cycle. As long as flumazenil-discriminating monkeys responded $\geq 80\%$ on the flumazenil lever during the first cycle or midazolam-discriminating monkeys responded $\leq 20\%$ on the midazolam lever during the first cycle, dose-effect curves for midazolam or pregnanolone were generated on subsequent cycles with cumulative doses of test compound increasing by 0.25 log unit per cycle. When monkeys did not satisfy the above criterion on the first cycle, the session ended after that first cycle and testing did not occur again until monkeys satisfied the criteria for 2 consecutive sessions. In flumazenil-discriminating monkeys, increasing doses of a test compound were administered until monkeys responded $\leq 20\%$ on the flumazenil lever or rates were decreased to $< 10\%$ of control rates; in midazolam-discriminating monkeys, drug administration continued until monkeys responded $\geq 80\%$ on the midazolam lever or 4 shocks were delivered. For both groups, doses larger than 3.2 mg/kg of midazolam or 10 mg/kg of pregnanolone were not administered.

Drugs

The drugs used in these studies were midazolam hydrochloride (Bedford Laboratories, Bedford, OH), pregnanolone (Sternaloids, Inc., Newport, RI), flumazenil (gift from F.

Hoffmann LaRoche, Basel, Switzerland), and pentylenetetrazole (Sigma-Aldrich Co., St. Louis, MO). Midazolam was purchased as a commercially prepared solution and diluted with sterile water. Pregnanolone was dissolved in 45% (w/v) hydroxypropyl- γ -cyclodextrin. Flumazenil was dissolved in a vehicle comprising 40% propylene glycol, 50% sterile water, and 10% ethanol; pentylenetetrazole was dissolved in sterile water. Doses are expressed in terms of the forms listed above in mg/kg body weight. Drugs were administered s.c. in the back.

Data Analyses

Discrimination data are expressed as the average percentage of total responses emitted on the drug lever $\pm$ 1 S.E.M. and plotted as a function of dose or time. Control response rates were determined when monkeys received only saline or sham injections and satisfied the testing criteria during each cycle of the session. A mean response rate for each session was calculated by averaging response rates for each cycle; control response rates represent the average of the means for 5 training sessions. Discrimination data were not included in the analyses when a subject responded at a rate less than 10% of its saline control rate.

Doses of positive modulators needed to produce 50% responding on the drug lever (ED_{50}) were estimated by fitting straight lines to individual dose-effect curves for each monkey; each line was estimated using one dose that produced not more than 25% responding on the drug lever, one dose that produced at least 75% responding on the drug lever and all doses in between. Potency ratios were calculated for each animal by dividing ED_{50} values in the presence of the large dose of flumazenil or pentylenetetrazole by ED_{50} values in the presence of the smaller dose of the same drug. Significant changes in potencies were detected when the 95% confidence intervals of the potency ratios averaged among monkeys did not include 1.

Results

Response rates (mean $\pm$ 1 S.E.M.) in four diazepam-treated monkeys that discriminated flumazenil while responding under a fixed-ratio 5 schedule of food presentation were 0.94 ± 0.08 , 1.18 ± 0.04 , 0.88 ± 0.08 and 0.70 ± 0.04 responses/s. Rates were higher in untreated monkeys that discriminated midazolam and responded under a fixed-ratio 10 schedule of stimulus-shock termination: 2.48 ± 0.11 , 3.03 ± 0.19 , 2.70 ± 0.13 and 1.63 ± 0.24 responses/s. When flumazenil-discriminating monkeys received 5.6 mg/kg of diazepam 3 h before sessions and vehicle during sessions, they responded on the vehicle lever; similarly, when midazolam-discriminating monkeys received vehicle during sessions, they responded predominantly on the vehicle lever (data not shown).

In diazepam-treated monkeys, flumazenil and pentylenetetrazole dose-dependently increased flumazenil-lever responding (Figure 1). The smallest doses that produced predominantly drug-lever responding were 0.1 mg/kg of flumazenil and 32 mg/kg of pentylenetetrazole. These effects were evident within 15 min and began to decrease 90 min later. Doses that were 0.25 log unit smaller produced 65-80% flumazenil-lever responding 15 min after administration and these effects were markedly diminished 90 min after administration.

In untreated monkeys, midazolam dose-dependently increased responding on the midazolam lever with monkeys responding predominantly on the drug lever at 0.1 mg/kg (circles, upper panel, Figure 2). Flumazenil produced responding exclusively on the saline lever (points above Flu, upper panel, Figure 2), and the smallest dose of flumazenil (0.01 mg/kg) antagonized the discriminative stimulus effects of midazolam, shifting the midazolam dose-effect curve to the right. A larger dose of flumazenil shifted the curve 2.0-fold further to the

right (Table 1; compare triangles and squares, upper panel, Figure 2). When studied in combination with midazolam in diazepam-treated monkeys, a dose of 0.1 mg/kg of flumazenil was needed to produce responding predominantly on the flumazenil lever; that effect was reversed by midazolam (diamonds, lower panel, Figure 2). Increasing the flumazenil dose to 0.178 mg/kg resulted in a 2.6-fold shift to the right in the midazolam dose-effect curve (Table 2; compare diamonds and inverted triangles, lower panel, Figure 2). Doses of midazolam larger than 3.2 mg/kg were not administered, although a dose of 0.32 mg/kg of flumazenil appeared to shift the midazolam dose-effect curve further to the right (lower panel, Figure 2).

Pregnanolone also produced midazolam-lever responding in untreated monkeys discriminating midazolam with a dose of 5.6 mg/kg producing 100% responding on the drug lever (circles, upper panel, Figure 3). The interaction between flumazenil and pregnanolone was qualitatively different from the interaction between flumazenil and midazolam. Specifically, flumazenil enhanced the discriminative stimulus effects of pregnanolone, with 0.1 mg/kg shifting the pregnanolone dose-effect curve 2.2-fold to the left (diamonds, upper panel, Figure 3). When the dose of flumazenil was increased, there was no further shift in the pregnanolone dose-effect curve (Table 1; compare diamonds and inverted triangles, upper panel, Figure 3). In diazepam-treated monkeys, pregnanolone reversed flumazenil-lever responding that was established by a dose of 0.1 mg/kg of flumazenil (diamonds, lower panel, Figure 3). Increasing the dose of flumazenil to 0.178 mg/kg did not change the potency of pregnanolone to reverse flumazenil-lever responding (Table 2; compare diamonds and inverted triangles, lower panel, Figure 3).

Pentylenetetrazole attenuated the discriminative stimulus effects of both positive modulators. In untreated monkeys discriminating midazolam, pentylenetetrazole did not occasion responding on the midazolam lever (points above PTZ, upper panel, Figure 4). A dose of 17.8 mg/kg of pentylenetetrazole shifted the midazolam dose-effect to the right, with 32 mg/kg of pentylenetetrazole producing a 2.1-fold further shift in the midazolam dose-effect curve (Table 1; compare triangles and squares, upper panel, Figure 4). In diazepam-treated monkeys, a dose of 32 mg/kg of pentylenetetrazole produced flumazenil-lever responding (points above PTZ, lower panel, Figure 4) and this effect was reversed by midazolam (squares, lower panel, Figure 4). Increasing the dose of pentylenetetrazole resulted in a 4.1-fold shift to the right in the midazolam dose-effect curve (Table 2; compare diamonds and squares, lower panel, Figure 4).

Pentylenetetrazole also attenuated the discriminative stimulus effects of pregnanolone in both groups of monkeys. In untreated monkeys discriminating midazolam, a dose of 17.8 mg/kg of pentylenetetrazole did not alter the pregnanolone dose-effect curve; however, a dose of 32 mg/kg of pentylenetetrazole shifted the pregnanolone dose-effect curve 2.3-fold to the right (Table 1; compare triangles and squares, upper panel, Figure 5). In diazepam-treated monkeys, pregnanolone reversed the discriminative stimulus effects of pentylenetetrazole. Increasing the dose of pentylenetetrazole shifted the pregnanolone dose-effect curve 2.7-fold to the right (Table 2; compare squares and diamonds, lower panel, Figure 5).

Discussion

Chronic benzodiazepine treatment can result in the development of dependence and tolerance to most effects of benzodiazepines. In vitro, GABA_A receptors are altered by chronic treatment; however, any contribution of these changes to the development of tolerance or dependence is not yet established. In vivo, differences in the behavioral effects of benzodiazepines and neuroactive steroids are evident when benzodiazepines are

administered chronically; tolerance develops to the discriminative stimulus and rate-decreasing effects of benzodiazepines and cross tolerance does not develop to neuroactive steroids (McMahon et al. 2007; Gerak 2009). If differential changes observed in vitro contribute to differences in the behavioral effects of positive modulators observed in vivo, then interactions between drugs in untreated monkeys might not predict interactions between the same drugs in diazepam-treated monkeys.

In untreated monkeys discriminating midazolam, flumazenil antagonized the discriminative stimulus effects of midazolam and not those of pregnanolone. In fact, the pregnanolone dose-effect curve was shifted 2.2-fold to the left following administration of 0.1 mg/kg of flumazenil, further supporting the notion that flumazenil has low-efficacy positive modulatory effects in rhesus monkeys (McMahon and France 2006). Although pentylenetetrazole does not act at either the benzodiazepine or neuroactive steroid sites, it is a negative modulator and it attenuated the effects of positive modulators acting at either site. In order to study positive modulators in diazepam-treated monkeys discriminating flumazenil, an effect was first established with either flumazenil or pentylenetetrazole which was then reversed by the positive modulators; consequently, dose-effect curves for the discriminative stimulus effects of midazolam and pregnanolone were not determined in the absence of flumazenil or pentylenetetrazole. In diazepam-treated monkeys, the doses of flumazenil needed to produce flumazenil-lever responding were 6- to 10-fold larger than those needed to antagonize the discriminative stimulus effects of midazolam in untreated monkeys. Both midazolam and pregnanolone reversed flumazenil-lever responding that was established with either flumazenil or pentylenetetrazole. Because flumazenil-lever responding is likely one sign of benzodiazepine withdrawal (McMahon et al. 2007), these results confirm that positive modulators acting at either the benzodiazepine or neuroactive steroid sites can reverse withdrawal.

To determine whether drug interactions are altered as a result of chronic diazepam treatment, changes in dose-effect curves for positive modulators obtained in combination with different doses of flumazenil or pentylenetetrazole were compared between the two groups of monkeys. For simple, competitive interactions, such as those between flumazenil and benzodiazepines, larger doses of flumazenil should further shift the benzodiazepine dose-effect curve to the right (Paronis and Bergman 1999; Lelas et al. 2000), and when the flumazenil dose increased by 0.25 log unit, the midazolam dose-effect curve was shifted 2-fold further to the right in both groups of monkeys. In the current study, noncompetitive interactions also did not differ as a result of chronic treatment. For example, increasing the dose of flumazenil did not shift the pregnanolone dose-effect curve in either group of monkeys. The ability of flumazenil to attenuate the discriminative stimulus effects of midazolam similarly in untreated monkeys discriminating midazolam and in diazepam-treated monkeys discriminating flumazenil suggests that these actions of flumazenil at benzodiazepine sites are not altered as a result of repeated diazepam administration. That chronic treatment also does not change the interaction between flumazenil and pregnanolone suggests that this approach does not provide insight into which changes in GABA_A receptors might account for differences in the behavioral effects of benzodiazepines and neuroactive steroids.

To further test whether changes in binding sites during chronic diazepam treatment can be predicted by drug interactions in vivo, a negative modulator that does not act at either the benzodiazepine or neuroactive steroid sites was studied in combination with each positive modulator in both groups. Because of its negative efficacy, pentylenetetrazole was expected to attenuate the effects of positive modulators, regardless of their site of action. In both groups of monkeys, pentylenetetrazole antagonized the discriminative stimulus effects of midazolam and pregnanolone. Shifts to the right in dose-effect curves increased comparably

across all conditions as the dose of pentylenetetrazole increased, further supporting the notion that changes in the benzodiazepine or neuroactive steroid binding sites do not translate to fundamental changes in interactions among drugs.

In vitro studies have provided several possibilities, other than changes in the modulatory sites, that might account for differences observed between benzodiazepines and neuroactive steroids during chronic benzodiazepine treatment. For example, benzodiazepine sites become functionally uncoupled from GABA sites during chronic treatment, which could be due to phosphorylation of GABA_A receptors or associated proteins, internalization of GABA_A receptors, changes in subunit composition, or a combination of these mechanisms (Tietz et al. 1989; Hu and Ticku 1994b). Some of these mechanisms could potentially result in differences between benzodiazepines and neuroactive steroids by perhaps reducing the number, but not the function, of benzodiazepine modulatory sites without altering the neuroactive steroid sites. For example, modulatory sites are formed when particular subunits are present, and the subunits that are needed to form each site differ (Puia et al. 1991; Hosie et al. 2006); changes in certain subunits (e.g., α) could alter the number of benzodiazepine sites and have no impact on neuroactive steroid sites.

In summary, interactions between GABA_A modulators acting at the same or different modulatory sites do not change as a consequence of chronic benzodiazepine treatment in these drug discrimination procedures. Although changes in GABA_A receptors have been identified in vitro, either those changes do not occur in vivo or they do not appear to impact interactions between modulators acting at different sites. It is unlikely that procedural differences (e.g., reinforcer type) between the two groups of monkeys contribute to the effects observed in the current study; in previous studies, such variables have been shown to have little impact on results obtained in these monkeys (Gerak and France 1999). Further study of the consequences of chronic benzodiazepine treatment as well as the differences between positive GABA_A modulators is necessary to understand changes in GABA_A receptor function resulting from chronic benzodiazepine treatment.

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Figure 1.

Time course for the discriminative stimulus effects of flumazenil (left panel) and pentylentetrazole (right panel) in four diazepam-treated monkeys discriminating flumazenil. Horizontal axes: time (hr) since drug administration. Vertical axes: mean (± 1 SEM) percentage of total responding that occurred on the flumazenil-appropriate lever.

Figure 2.

Discriminative stimulus effects of midazolam studied in combination with flumazenil in untreated monkeys discriminating midazolam (upper panel) and in diazepam-treated monkeys discriminating flumazenil (lower panel). Midazolam was also studied in the absence of flumazenil in untreated monkeys. Horizontal axes: dose (mg/kg body weight); data above Flu represent the effects of flumazenil determined on the first cycle immediately before determination of the midazolam dose-effect curve. Vertical axes, upper panel: mean (± 1 SEM) percentage of total responding that occurred on the midazolam-appropriate lever; lower panel: mean (± 1 SEM) percentage of total responding that occurred on the flumazenil-appropriate lever.

Figure 3.

Discriminative stimulus effects of pregnanolone studied in combination with flumazenil in untreated monkeys discriminating midazolam (upper panel) and in diazepam-treated monkeys discriminating flumazenil (lower panel). Pregnanolone was also studied in the absence of flumazenil in untreated monkeys. Horizontal axes: dose (mg/kg body weight); data above Flu represent the effects of flumazenil determined on the first cycle immediately before determination of the midazolam dose-effect curve. Vertical axes, upper panel: mean (± 1 SEM) percentage of total responding that occurred on the midazolam-appropriate lever; lower panel: mean (± 1 SEM) percentage of total responding that occurred on the flumazenil-appropriate lever.

Figure 4.

Discriminative stimulus effects of midazolam studied in combination with pentylenetetrazole in untreated monkeys discriminating midazolam (upper panel) and in diazepam-treated monkeys discriminating flumazenil (lower panel). Midazolam was also studied in the absence of pentylenetetrazole in untreated monkeys. Horizontal axes: dose (mg/kg body weight); data above PTZ represent the effects of pentylenetetrazole determined on the first cycle immediately before determination of the midazolam dose-effect curve. Vertical axes, upper panel: mean (± 1 SEM) percentage of total responding that occurred on the midazolam-appropriate lever; lower panel: mean (± 1 SEM) percentage of total responding that occurred on the flumazenil-appropriate lever.

Figure 5.

Discriminative stimulus effects of pregnanolone studied in combination with pentylenetetrazole in untreated monkeys discriminating midazolam (upper panel) and in diazepam-treated monkeys discriminating flumazenil (lower panel). Pregnanolone was also studied in the absence of pentylenetetrazole in untreated monkeys. Horizontal axes: dose (mg/kg body weight); data above PTZ represent the effects of pentylenetetrazole determined on the first cycle immediately before determination of the midazolam dose-effect curve. Vertical axes, upper panel: mean ($\pm$ 1 SEM) percentage of total responding that occurred on the midazolam-appropriate lever; lower panel: mean ($\pm$ 1 SEM) percentage of total responding that occurred on the flumazenil-appropriate lever.

Table 1

Mean ED₅₀ values and potency ratios with 95% confidence intervals (CI) for the discriminative stimulus effects of midazolam and pregnanolone, in combination with flumazenil and pentylenetetrazole, in monkeys discriminating midazolam.

	ED ₅₀ (mg/kg)		Potency ratio ^a	
	Mean	95% CI	Mean	95% CI
Midazolam	0.061	0.037-0.084		
+0.01 mg/kg flumazenil	0.10	0.053-0.15		
+0.0178 mg/kg flumazenil	0.20	0.12-0.27	2.00*	1.02-2.99
+17.8 mg/kg pentylenetetrazole	0.13	0.08-0.17		
+32 mg/kg pentylenetetrazole	0.28	0.08-0.47	2.07*	1.19-2.94
Pregnanolone	2.80	2.13-3.48		
+0.1 mg/kg flumazenil	1.27	1.09-1.46		
+0.178 mg/kg flumazenil	1.44	1.06-1.82	1.16	0.65-1.66
+17.8 mg/kg pentylenetetrazole	3.05	1.41-4.70		
+32 mg/kg pentylenetetrazole	6.57	3.67-9.46	2.25*	1.15-3.35

^aPotency ratio of ED₅₀ values determined in the presence of the large dose of flumazenil or pentylenetetrazole divided by ED₅₀ values determined in the presence of the smaller dose of the same drug.

* Significant changes in potency of the positive modulator when the dose of flumazenil or pentylenetetrazole was increased.

Table 2

Mean ED₅₀ values and potency ratios with 95% confidence intervals (CI) for the discriminative stimulus effects of midazolam and pregnanolone, in combination with flumazenil and pentyleneetetrazole, in monkeys receiving diazepam daily and discriminating flumazenil.

	ED ₅₀ (mg/kg)		Potency ratio ^a	
	Mean	95% CI	Mean	95% CI
Midazolam				
+0.1 mg/kg flumazenil	0.80	0.65-0.95		
+0.178 mg/kg flumazenil	2.05	1.15-2.95	2.56*	1.51-3.61
+32 mg/kg pentyleneetetrazole	0.51	0.05-0.96		
+56 mg/kg pentyleneetetrazole	1.79	0.88-2.70	4.05*	2.00-6.10
Pregnanolone				
+0.1 mg/kg flumazenil	2.50	1.90-3.11		
+0.178 mg/kg flumazenil	2.60	1.96-3.24	1.04	0.97-1.11
+32 mg/kg pentyleneetetrazole	0.68	0.24-1.10		
+56 mg/kg pentyleneetetrazole	1.69	0.84-2.53	2.74*	1.18-4.30

^a Potency ratio of ED₅₀ values determined in the presence of the large dose of flumazenil or pentyleneetetrazole divided by ED₅₀ values determined in the presence of the smaller dose of the same drug.

* Significant changes in potency of the positive modulator when the dose of flumazenil or pentyleneetetrazole was increased.