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Acute cross tolerance to midazolam, and not pentobarbital and pregnanolone, following a single dose of chlordiazepoxide in monkeys discriminating midazolam

Lisa R. Gerak, Lance R. McMahon, and Charles P. France

Departments of Pharmacology (LRG, LRM and CPF) and Psychiatry (CPF), The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, mail code 7764, San Antonio, TX, USA 78229-3900

Abstract

Although cross tolerance can develop among positive GABA_A modulators acting at the same modulatory site, cross tolerance does not always develop to drugs acting at sites that are different from the site of action of the drug administered chronically. To examine the relationship between cross tolerance and site of action, four rhesus monkeys discriminated midazolam and, on separate occasions, received 32 mg/kg of chlordiazepoxide 24 hr before dose-effect determinations for drugs acting at different sites. Midazolam, pentobarbital and pregnanolone produced >80% midazolam-lever responding. Although monkeys responded on the midazolam lever 2–4 hr after 32 mg/kg of chlordiazepoxide, they responded on the saline lever 24 hr later. Twenty-four hr after an acute injection of 32 mg/kg of chlordiazepoxide, midazolam dose-effect curves were shifted 4.6-fold to the right whereas pregnanolone dose-effect curves were shifted 3-fold to the left. Sensitivity to pentobarbital increased in one monkey and decreased in others 24 hr after chlordiazepoxide. Decreased sensitivity to midazolam demonstrates that acute cross tolerance develops following chlordiazepoxide administration, although it does not develop to drugs acting at other sites. These differences among positive GABA_A modulators suggest that even short-term benzodiazepine administration changes GABA_A receptors, and those changes impact modulatory sites differently.

Keywords

acute tolerance; chlordiazepoxide; pregnanolone; pentobarbital; neuroactive steroids; monkeys; drug discrimination

Introduction

There are several modulatory sites on γ -aminobutyric acid_A (GABA_A) receptors through which drugs can facilitate the actions of GABA. Positive GABA_A modulators that act at benzodiazepine and barbiturate modulatory sites are used clinically to treat disorders such as anxiety and insomnia. Drugs acting at a third site on GABA_A receptors, the neuroactive steroid site, have not been used clinically, although under many conditions their effects appear to be similar to those produced by positive modulators acting at other sites. Similarities among positive GABA_A modulators in vitro (Majewska et al. 1986; Allan and Harris 1986; Obata et al. 1988) seem to confer similarities in their effects in vivo, including anxiolytic, sedative and anticonvulsant effects (Wieland et al. 1997; Vanover et al. 1999; Reddy and Rogawski

2001); however, actions at distinct binding sites appear to result in some effects that are not identical.

One procedure in which positive GABA_A modulators produce similar but not identical effects is drug discrimination. Positive GABA_A modulators acting at benzodiazepine, barbiturate or neuroactive steroid modulatory sites have been trained as discriminative stimuli, and generally, although not always, they share discriminative stimulus effects (e.g., de la Garza and Johanson 1987; Ator et al. 1993; McMahon et al. 2001; Engel et al. 2001). Differences among positive GABA_A modulators have been reported under several conditions. For example, phenobarbital and pentobarbital produce drug-lever responding in only a subset of pigeons discriminating midazolam (Evans and Johanson 1989), and pentobarbital does not produce lorazepam-lever responding in rats or baboons (Ator and Griffiths 1983; Ator et al. 1993). Thus, given the high pharmacological selectivity of drug discrimination procedures, differences in discriminative stimulus effects suggest that positive GABA_A modulators acting at different sites are not identical.

Another apparent difference among positive GABA_A modulators occurs during chronic treatment. Specifically, although tolerance to benzodiazepines can be readily established in the laboratory, tolerance to neuroactive steroids is much more difficult to demonstrate and does not develop under many conditions (Kokate et al. 1998; Reddy and Rogawski 2000; Damianisch et al. 2001; McMahon and France 2002a). In addition, during chronic benzodiazepine treatment, sensitivity to benzodiazepines decreases, indicating the development of tolerance or cross tolerance, whereas sensitivity to neuroactive steroids does not appear to change (Czlonkowska et al. 2001; McMahon and France 2002b; McMahon et al. 2007). This differential development of tolerance and cross tolerance to positive GABA_A modulators suggests that changes in GABA_A receptors during chronic treatment impact modulatory sites differently. A better understanding of these effects might provide new insight into GABA_A receptor function.

In order to further investigate differences among positive GABA_A modulators, the current study evaluated changes in sensitivity to drugs acting at different modulatory sites before and after administration of the benzodiazepine chlordiazepoxide in rhesus monkeys discriminating the benzodiazepine midazolam. This study tested the generality of effects obtained during chronic benzodiazepine treatment (e.g., Czlonkowska et al. 2001; McMahon and France 2002b; McMahon et al. 2007) by examining acute cross tolerance to positive GABA_A modulators (Barrett and Smith 2005). Although long-term benzodiazepine treatment clearly produces cross tolerance to positive modulators acting at benzodiazepine sites and not those acting at neuroactive steroid sites (Czlonkowska et al. 2001; McMahon and France 2002b), it is not clear whether much shorter periods of treatment also alter potency of positive modulators differentially depending on their site of action.

Methods

Subjects

Three female and one male (subject GI) adult rhesus monkeys weighed between 4.0 and 8.0 kg and were housed individually on a 14 hr light and 10 hr dark cycle. Monkeys had unlimited access to water and were maintained at their free-feeding weights with primate chow (Harlan Teklad, High Protein Monkey Diet, Madison, WI), fresh fruit and peanuts provided in the home cage. They had been trained to discriminate midazolam at least one year before the start of these studies (McMahon and France 2005). 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

During experimental sessions, subjects were seated in chairs (Primate Products, Miami, FL) and placed within ventilated, sound-attenuating chambers. Each chamber was equipped with two stimulus lights and two response levers. The feet of monkeys were placed in shoes that were mounted to the front of chairs. Shoes contained brass electrodes to which brief (250 msec, 3mA) electric shock could be delivered from an a.c. shock generator located outside chambers. A commercially available interface (Med Associates Inc., East Fairfield, VT, USA) connected experimental chambers to computers which controlled experiments and recorded data.

Procedure

Monkeys discriminated a dose of 0.32 mg/kg of midazolam while responding under a fixed-ratio 10 schedule of stimulus-shock termination (Lelas et al. 1999; McMahon and France 2005). For one monkey (subject NI), the training dose of midazolam was decreased to 0.178 mg/kg during these studies because the larger dose decreased response rate. Daily experimental sessions were 30 to 120 min in length with each session divided into 15-min cycles. The first 10 min of cycles were timeout periods during which chambers were dark and responding had no programmed consequence. The last 5 min of cycles were response periods during which the schedule of stimulus-shock termination was in effect; the beginning of response periods was signaled by illumination of red stimulus lights located above each lever. When red lights were illuminated during training sessions, monkeys could extinguish stimulus lights and postpone the shock schedule for 30 sec by responding 10 consecutive times (fixed-ratio 10) on the lever designated correct by an injection administered during the first minute of the cycle. Following midazolam administration, the left lever was designated correct for some monkeys, and the right lever was designated correct for other monkeys. Incorrect responses reset the fixed-ratio requirement on the correct lever. If monkeys did not satisfy the fixed-ratio requirement within 15 sec of illumination of red lights, brief electric shock was delivered. Thereafter, shock was delivered every 15 sec until the response requirement was satisfied, the cycle ended, or four shocks were delivered, whichever occurred first.

During training sessions, midazolam, saline or sham injections were administered during the first minute of each cycle. On some occasions, monkeys received saline or sham injections (i.e., monkeys were handled and did not receive an injection) prior to all cycles, with the number of cycles varying between 2 and 8. On other occasions, monkeys received the training dose of midazolam during one cycle followed by a single sham injection during the next cycle; responding on the drug-appropriate lever extinguished lights and postponed the shock schedule during cycles in which midazolam was administered and the subsequent sham cycle, which ended the session. The cycle during which midazolam 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), and for these 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 10 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 of three consecutive sessions.

Test sessions were identical to training sessions except that 10 consecutive responses on either lever postponed shock and various doses of test compounds were administered either immediately before sessions or during the first minute of each cycle during sessions. Initially, the time course of the discriminative stimulus effects of chlordiazepoxide was studied by

administering a single dose (3.2–56 mg/kg) either immediately or 2 hr before sessions comprising 8 sham cycles. To determine whether the discriminative stimulus effects of chlordiazepoxide were evident the day after administration, two sham cycles were conducted 24 hr after each dose of chlordiazepoxide; a dose of 32 mg/kg was the largest dose that elicited predominantly saline-lever responding 24 hr later and that dose was used thereafter to examine the possible development of acute cross tolerance. Each of these experiments was conducted over three consecutive days. On the first day, a dose-effect curve was determined for midazolam, pregnanolone or pentobarbital using a cumulative-dosing procedure. Vehicle was administered during the first minute of the first cycle of test sessions. On subsequent cycles, increasing doses of test compound were administered with the cumulative dose increasing by 0.25 or 0.5 log unit per cycle. Dosing continued until monkeys responded $\geq 80\%$ on the midazolam lever or 4 shocks were delivered; however, doses of pentobarbital larger than 17.8 mg/kg and doses of pregnanolone larger than 10 mg/kg were not administered to avoid adverse effects. If a drug did not produce $\geq 80\%$ responding on the midazolam lever, then no further testing was done with that drug in that monkey. Dose-effect curves for drugs that produced $\geq 80\%$ responding on the midazolam lever in a particular monkey were determined 24 hr before and 24 hr after administration of chlordiazepoxide. On separate occasions, 3.2 and 32 mg/kg of chlordiazepoxide were given to examine acute cross tolerance to midazolam. Because 3.2 mg/kg did not produce effects alone or in combination with midazolam, only 32 mg/kg was studied with pentobarbital and pregnanolone. Chlordiazepoxide was administered during the first min of a session comprising 8 cycles. Subsequent tests were not conducted for at least one week after monkeys received chlordiazepoxide, and only when stimulus control was reestablished with midazolam.

Drugs

The drugs used in these studies were midazolam hydrochloride (Bedford Laboratories, Bedford, OH), pregnanolone (Steraloids, Inc., Newport, RI), pentobarbital sodium and chlordiazepoxide hydrochloride (Sigma-Aldrich Co., St. Louis, MO). Pregnanolone was dissolved in 45% (w/v) hydroxypropyl- γ -cyclodextrin. Pentobarbital and chlordiazepoxide were dissolved in sterile water; midazolam was purchased as a commercially prepared solution and diluted with 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 midazolam lever ± 1 S.E.M. and plotted as a function of dose. 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.

Differences between dose-effect curves determined before and after administration of chlordiazepoxide were analyzed by fitting straight lines to dose-effect curve data using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA) and the following equation: $\text{effect} = \text{slope} \cdot \log(\text{dose}) + \text{intercept}$. Dose-effect curves generated before and after chlordiazepoxide administration were compared using mathematical models that varied in complexity. Simpler models had common parameters (common slope, common intercept) whereas more complex models allowed slopes and intercepts to vary for each dose-effect curve; simple models were compared to complex models by means of an *F*-ratio test. If the calculated *F* value was significant, then the more complex model was required to fit the

data. If the calculated F value was not significant, the simpler model was used (Kenakin 1997).

Results

In the absence of drug, response rates (mean $\pm$ 1 S.E.M.) in four monkeys responding under a fixed-ratio 10 schedule of stimulus-shock termination were 2.48 ± 0.11 responses/s for subject RO, 3.03 ± 0.19 responses/s for subject SA, 2.70 ± 0.13 responses/s for subject NI, and 1.63 ± 0.24 responses/s for subject GI. When monkeys received saline, they responded on the saline-appropriate lever and response rates were similar to control rates (points above V, Figure 1). Midazolam dose-dependently increased responding on the midazolam-appropriate lever with all monkeys responding $>80\%$ on the drug lever at 0.32 mg/kg (upper left panel, Figure 1); that dose decreased responding to $<40\%$ of control in two of the four monkeys (lower left panel, Figure 1). Positive GABA_A modulators acting at other modulatory sites produced midazolam-lever responding in some but not all monkeys. A dose of 17.8 mg/kg of pentobarbital produced midazolam-lever responding and decreased response rates in three of the four monkeys; neither effect was obtained in the fourth monkey (subject RO) and larger doses were not studied to avoid adverse effects (middle panels, Figure 1). Pregnanolone also produced midazolam-lever responding in three of four monkeys, although doses required to produce $>80\%$ responding on the midazolam lever ranged from 3.2 to 10 mg/kg; the fourth monkey (subject SA) did not respond on the midazolam lever up to doses of pregnanolone that markedly decreased responding (right panels, Figure 1).

Chlordiazepoxide dose and time dependently increased responding on the midazolam lever. The discriminative stimulus effects of small doses of chlordiazepoxide were not evident until two hours after administration, and after 10 or 32 mg/kg, the largest percentage of responding on the midazolam lever was 67% (upper panel, Figure 2). The onset of action was more rapid for a larger dose of chlordiazepoxide with monkeys responding $>80\%$ on the midazolam lever 30 min after administration of 56 mg/kg (diamonds, upper panel, Figure 2). The discriminative stimulus effects of chlordiazepoxide were diminished after 24 hr with the smaller doses producing predominantly saline-lever responding and 56 mg/kg producing 40% midazolam-lever responding (rightmost points, upper panel, Figure 2). Doses of 10 and 32 mg/kg of chlordiazepoxide had modest rate-decreasing effects whereas 56 mg/kg generally had more dramatic effects with responding reduced to $<70\%$ of control at most time points; rate-decreasing effects were not evident 24 hr after chlordiazepoxide administration (rightmost points, lower panel, Figure 2).

Sensitivity to midazolam decreased 24 hr after administration of chlordiazepoxide. A small dose of chlordiazepoxide (3.2 mg/kg), which did not produce midazolam-lever responding for up to 4 hr after administration (data not shown), did not alter sensitivity to midazolam (upper panel, Figure 3). Dose-effect curves determined 24 hr before and 24 hr after chlordiazepoxide could be fitted with a common slope ($F_{(1,17)}=0.340$, $P>0.05$) and intercept ($F_{(1,18)}=2.086$, $P>0.05$), indicating that they were not different. A larger dose (32 mg/kg) of chlordiazepoxide administered 24 hr earlier decreased sensitivity to midazolam, as indicated by a 4.6-fold shift to the right in the midazolam dose-effect curve (squares, upper panel, Figure 3). The simplest model that could be fitted to these data had a common slope ($F_{(1,28)}=0.858$, $P>0.05$) but a significantly different intercept ($F_{(1,29)}=9.073$, $P<0.01$), indicating that this dose of chlordiazepoxide significantly decreased the potency of midazolam. Larger doses of chlordiazepoxide were not studied because the discriminative stimulus effects of 56 mg/kg of chlordiazepoxide were still evident 24 hr after administration. Sensitivity to the rate-decreasing effects of midazolam was not changed by either dose of chlordiazepoxide (lower panel, Figure 3).

Sensitivity to pentobarbital also changed following chlordiazepoxide administration (Figure 4); unlike effects obtained with midazolam, changes in sensitivity to pentobarbital were qualitatively different among monkeys. Although pentobarbital produced >80% responding on the drug lever only on 2 of the 3 occasions in which it was studied in subject NI (see figure 1), it produced >80% responding on the midazolam lever on the day before chlordiazepoxide was administered. In one monkey (subject GI), administration of 32 mg/kg of chlordiazepoxide 24 hr earlier increased sensitivity to pentobarbital, shifting the pentobarbital dose-effect curve 2-fold to the left. In the other two monkeys, including subject NI, sensitivity to pentobarbital decreased, although the magnitude of this change could not be determined because doses of pentobarbital larger than 17.8 mg/kg were not studied to avoid adverse effects. Nevertheless, a dose of 17.8 mg/kg of pentobarbital, which produced >80% midazolam-lever responding 24 hr before chlordiazepoxide administration, produced <20% midazolam-lever responding in those 2 monkeys 24 hr after 32 mg/kg of chlordiazepoxide administration. The simplest model to which these data could be fitted had a common slope ($F_{(1,14)}=2.037$, $P>0.05$) and intercept ($F_{(1,15)}=1.019$, $P>0.05$). Chlordiazepoxide did not markedly alter sensitivity to the rate-decreasing effects of pentobarbital (lower panel, Figure 4).

The effect of chlordiazepoxide on sensitivity to pregnanolone was different from its effect on sensitivity to either midazolam or pentobarbital. A dose of 32 mg/kg of chlordiazepoxide, administered 24 hr earlier, increased sensitivity to pregnanolone, producing a 3-fold shift to the left in the pregnanolone dose-effect curve, as compared to the dose-effect curve determined 24 hr before chlordiazepoxide administration (upper panel, Figure 5). These data could be fitted with a common slope ($F_{(1,17)}=0.595$, $P>0.05$) but with different intercepts ($F_{(1,18)}=14.55$, $P<0.01$), indicating that chlordiazepoxide significantly increased the potency of pregnanolone. Although chlordiazepoxide had modest rate-decreasing effects 24 hr after administration (point above V, lower panel, Figure 5), it did not alter the rate-decreasing effects of pregnanolone (lower panel, Figure 5).

Discussion

Benzodiazepine treatment can result in the development of tolerance as well as cross tolerance to other benzodiazepines; this decreased sensitivity to benzodiazepines can occur following acute (Khanna et al. 1998) or chronic treatment with chlordiazepoxide (Le et al. 1986; Sannerud et al. 1993). Similar effects were obtained in the current study; acute cross tolerance to midazolam developed in monkeys that received chlordiazepoxide 24 hr earlier. Acute cross tolerance to positive GABA_A modulators acting at other sites has not been established, although chronic benzodiazepine treatment does not appear to confer cross tolerance to neuroactive steroids (Czlonkowska et al. 2001; McMahon and France 2002b; McMahon et al. 2007). In the current study, acute cross tolerance did not develop to pregnanolone under conditions that decreased sensitivity to midazolam. Thus, long-term benzodiazepine treatment is not required to demonstrate that the development of cross tolerance varies depending on site of action of positive GABA_A modulators, and the consistency of results obtained in the current study and those obtained during chronic benzodiazepine treatment further extends the conditions under which such differences occur among positive GABA_A modulators.

Decreased sensitivity to pregnanolone, which would be indicative of acute cross tolerance, was not evident 24 hr after chlordiazepoxide administration. In fact, under those treatment conditions, sensitivity to pregnanolone increased 3-fold. Decrease sensitivity to benzodiazepines with concomitant enhanced sensitivity to neuroactive steroids has also been observed in monkeys receiving diazepam chronically (McMahon and France 2002b; McMahon et al. 2007), and these similarities in effects obtained following acute and chronic treatment suggest that the same mechanism might be responsible for these changes under both treatment conditions. There are several possible explanations for these qualitatively different effects

between benzodiazepines and neuroactive steroids. First, given the long duration of action observed for chlordiazepoxide, it is likely that chlordiazepoxide or its active metabolites are not entirely eliminated 24 hr after administration; although the amount of chlordiazepoxide remaining at this time is not sufficient to produce midazolam-lever responding, it might be sufficient to enhance the discriminative stimulus effects of pregnanolone. Moreover, if an additive effect between pregnanolone and any remaining chlordiazepoxide and its active metabolites accounts for the shift to the left in the pregnanolone dose-effect curve, then there would also be an additive effect between chlordiazepoxide and midazolam. Thus, the potency of midazolam 24 hr after chlordiazepoxide administration could be the result of two opposing effects: the development of acute cross tolerance, which decreases potency, and an additive effect, which increases potency. Decreased sensitivity to midazolam that was observed in this study might underestimate the magnitude of acute cross tolerance that occurs at benzodiazepine receptors after a single dose of chlordiazepoxide.

Another explanation for the qualitatively different changes in sensitivity to pregnanolone and midazolam could be related to changes in GABA_A receptors as a result of chlordiazepoxide treatment. One possible mechanism by which benzodiazepine tolerance and cross tolerance develops involves functional uncoupling of the benzodiazepine site from GABA and barbiturate sites during chronic treatment (Tietz et al. 1989; Hu and Ticku 1994a). The mechanism that induces uncoupling has not been established and is likely complex, possibly involving phosphorylation of GABA_A receptors or associated proteins, internalization of GABA_A receptors, changes in subunit composition, or some combination of these mechanisms (Friedman et al. 1996; Ali and Olsen 2001; Biggio et al. 2003). Some of these mechanisms could potentially result in qualitatively different effects during benzodiazepine treatment, like those observed in the current study. For example, because different subunits of GABA_A receptors are important in forming different modulatory sites (Puia et al. 1991; Hosie et al. 2006), changes in subunit composition could decrease the potency of benzodiazepines and increase potency of neuroactive steroids. In addition, chronic benzodiazepine treatment also appears to decrease allosteric coupling of neuroactive steroid and benzodiazepine sites (Friedman et al. 1996), which also could account for the qualitatively different changes in sensitivity to pregnanolone and midazolam observed in the current study.

Changes in sensitivity to pentobarbital are *less* consistent among subjects with the potency of pentobarbital increased slightly 24 hr after chlordiazepoxide administration only in one of the three monkeys. Changes in sensitivity to pentobarbital also might reflect the combination of two opposing effects, specifically additivity between pentobarbital and chlordiazepoxide that remains 24 hr after administration and acute cross tolerance to pentobarbital. This explanation is supported by effects obtained in monkey GI; this subject is the only monkey in which sensitivity to pentobarbital increased. Of the 3 monkeys in which pregnanolone was studied 24 hr after chlordiazepoxide, the largest increase in sensitivity was observed in monkey GI, with chlordiazepoxide producing a 6-fold shift to the left in the pregnanolone dose-effect curve; in addition, the smallest decrease in sensitivity to midazolam was observed in monkey GI. Thus, increased sensitivity to pentobarbital observed in this monkey might indicate that more chlordiazepoxide and its metabolites remain 24 hr after administration, as compared to the amount of chlordiazepoxide remaining in the other monkeys. Furthermore, differences in the magnitude of shift to the left of the pentobarbital and pregnanolone dose-effect curves in this monkey might also indicate that some cross tolerance developed to pentobarbital, and this hypothesis is supported by the decreased sensitivity to pentobarbital observed in the other two monkeys. Cross tolerance to barbiturates during benzodiazepine treatment is evident under some conditions (McMillan and Leander 1978; Le et al. 1986; McMillan 1992), although sensitivity to barbiturates does not always change during benzodiazepine treatment (Cesare and McKearney 1980; Sannerud et al. 1993; Khanna et al. 1998; McMahon and France 2002b). Chronic benzodiazepine treatment has also been shown to decrease the apparent

efficacy of pentobarbital in modifying GABA-mediated [$^{36}\text{Cl}^-$] flux, which might contribute to the development of cross tolerance; one mechanism that could account for these effects is uncoupling of benzodiazepine and barbiturate sites from GABA sites (Hu and Ticku 1994b). Because coupling of benzodiazepine sites to GABA and barbiturate sites is likely regulated independently of coupling to neuroactive steroids sites (Friedman et al. 1996), it is not surprising that decreased sensitivity to pentobarbital was observed in at least some monkeys 24 hr after chlordiazepoxide administration whereas sensitivity to pregnanolone was increased in all monkeys.

Some variability was also observed among subjects in the ability of positive GABA_A modulators to produce midazolam-lever responding. Generally, in subjects discriminating a positive modulator, benzodiazepines, barbiturates and neuroactive steroids produce drug-lever responding, regardless of the site of action of the training drug (de la Garza and Johanson 1987; Woudenberg and Slangen 1989; Vanover 1997; Lelas et al. 1999; Engel et al. 2001). Although benzodiazepines substitute for midazolam in all monkeys in the current study, drugs acting at other modulatory sites do not substitute in at least one monkey. Under other conditions, positive modulators acting at other, non-benzodiazepine sites do not substitute for a benzodiazepine discriminative stimulus (Ator and Griffiths 1983; Evans and Johanson 1989; Ator et al. 1993). These two-choice procedures in which subjects discriminate drug from its vehicle suggest that the discriminative stimulus effects of benzodiazepines and barbiturates are not identical; more complex discrimination procedures provide even more compelling evidence of these differences. When rats discriminate among a large dose of midazolam (3.2 mg/kg), a small dose of midazolam (0.32 mg/kg) and no drug, increasing doses of midazolam increase responding on the small-dose lever and then on the large-dose lever. In contrast, pentobarbital dose dependently increases responding on the small-dose lever only; larger doses do not produce responding on the lever associated with the large dose of midazolam (Sannerud and Ator 1995). In another type of drug discrimination procedure, subjects can discriminate chlordiazepoxide from pentobarbital (Barry and Krimmer 1979; Jarbe and Swedberg 1998). Although quantitative differences between these training conditions might partially account for their ability to discriminate chlordiazepoxide from pentobarbital, there is also evidence of qualitative differences between these drugs (Barry and Krimmer 1979). Together, results from these discrimination procedures suggest that the discriminative stimulus effects of benzodiazepines and barbiturates are not identical with factors such as training dose being important in identifying differences among positive GABA_A modulators. That pregnanolone and pentobarbital do not produce midazolam-lever responding in all monkeys in the current study is consistent with findings obtained under a variety of conditions.

In summary, acute cross tolerance to midazolam develops following administration of a single dose of chlordiazepoxide; however, acute cross tolerance to pregnanolone is not evident. Chronic benzodiazepine treatment has long been associated with changes in GABA_A receptors and more recently with differential development of cross tolerance to drugs acting at different modulatory sites. Results from the current study indicate that changes in GABA_A receptors can occur after short periods of benzodiazepine treatment and that these changes differentially impact actions of drugs at different modulatory sites. These differences among positive GABA_A modulators might provide critical insight into GABA_A receptor function and changes that occur during chronic treatment.

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Figure 1.

Discriminative stimulus and rate-decreasing effects of midazolam (left panels), pentobarbital (middle panels) or pregnanolone (right panels) in four monkeys discriminating midazolam. Data from individual monkeys are represented by different symbols; each symbol represents the average of three dose-effect curves determined on separate occasions in each monkey. Abscissae: dose in milligrams per kilogram body weight; data above V represent the effects of vehicle. Ordinates, upper panel: mean (± 1 SEM) percentage of total responding that occurred on the midazolam-appropriate lever; lower panel: average response rates expressed as a percentage of control rates (± 1 SEM).

Figure 2.

Discriminative stimulus and rate-decreasing effects of chlordiazepoxide administered immediately, 2 h or 24 hr before sessions in three monkeys discriminating midazolam. Each symbol represents the effects of different doses of chlordiazepoxide averaged among three monkeys. Abscissa: time in min following chlordiazepoxide administration. Ordinates, upper panel: mean (± 1 SEM) percentage of total responding that occurred on the midazolam-appropriate lever; lower panel: average response rates expressed as a percentage of control rates (± 1 SEM).

Figure 3.

Discriminative stimulus and rate-decreasing effects of midazolam determined 24 hr before or 24 hr after administration of 3.2 (n=3) or 32 (n=4) mg/kg of chlordiazepoxide in monkeys discriminating midazolam. Dose-effect curves were obtained before each dose of chlordiazepoxide; those two determinations were averaged for presentation. Abscissae: dose in milligrams per kilogram body weight; data above V represent the effects of vehicle. Ordinates, upper panel: mean (± 1 SEM) percentage of total responding that occurred on the midazolam-appropriate lever; lower panel: average response rates expressed as a percentage of control rates (± 1 SEM).

Figure 4.

Discriminative stimulus and rate-decreasing effects of pentobarbital determined 24 hr before and after administration of 32 mg/kg of chlordiazepoxide in the three monkeys in which pentobarbital produced >80% midazolam-lever responding. Abscissae: dose in milligrams per kilogram body weight; data above V represent the effects of vehicle. Ordinates, upper panel: mean (± 1 SEM) percentage of total responding that occurred on the midazolam-appropriate lever; lower panel: average response rates expressed as a percentage of control rates (± 1 SEM).

Figure 5.

Discriminative stimulus and rate-decreasing effects of pregnanolone determined 24 hr before and after administration of 32 mg/kg of chlordiazepoxide in the three monkeys in which pregnanolone produced >80% midazolam-lever responding. Abscissae: dose in milligrams per kilogram body weight; data above V represent the effects of vehicle. Ordinates, upper panel: mean (± 1 SEM) percentage of total responding that occurred on the midazolam-appropriate lever; lower panel: average response rates expressed as a percentage of control rates (± 1 SEM).