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Pharmacodynamic and receptor binding changes during chronic lorazepam administration

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Abstract

To assess pharmacodynamic and neurochemical aspects of tolerance, lorazepam (2 mg/kg/day), or vehicle was administered chronically to male Crl: CD-1(ICR)BR mice via implantable osmotic pump. Open-field behavior, benzodiazepine receptor binding in vitro, receptor autoradiography, and muscimol-stimulated chloride uptake were examined at both 1 and 14 days. Open-field activity was depressed in lorazepam-treated animals on Day 1. On Day 14, open-field parameters were indistinguishable from those of vehicle-treated animals, indicating behavioral tolerance. Benzodiazepine binding, as determined by the specific binding of [^{125}I]diazepam, was also decreased in cortex on Day 14. Hippocampal binding was unchanged following chronic lorazepam exposure. Apparent affinity in cortical membrane preparations was unchanged, indicating that altered ligand uptake was due to decreased receptor number. Muscimol-stimulated chloride uptake into cortical synaptoneurosomes from lorazepam-treated animals was not significantly different on Day 1 or Day 14 compared to vehicle-treated animals. These results confirm that down-regulation of benzodiazepine receptor binding is closely associated with behavioral tolerance to benzodiazepines. These observed changes in binding are not necessarily associated with robust changes in receptor function.

Keywords: Autoradiography; Behavior; Benzodiazepine; B_{max}; receptor density; GABA; γ-aminobutyric acid; K_d; apparent affinity

1. Introduction

Benzodiazepines continue to be the primary agents prescribed for the treatment of anxiety and insomnia and are widely used in the American population (Griffiths and Weerts, 1997; Shader and Greenblatt, 1993; Woods et al., 1992). Chronic administration of benzodiazepines is known to produce tolerance to many of the pharmacodynamic effects of these compounds in both animals and humans (Gonsalves and Gallager, 1986, 1987; Greenblatt et al., 1978; Lister et al., 1983; Miller et al., 1988a; Sievewright and Dougal, 1993; Treit, 1985; Woods et al., 1992). Although the neurochemical basis for the development of benzodiazepine tolerance remains uncertain, it has been associated with down-regulation of benzodiazepine binding and function of the $GABA_A$ receptor (Allan et al., 1992; Byrnes et al., 1993; Facklam et al., 1992; Gallager et al., 1984; Galpern et al., 1990; Hu and Ticku, 1994; Klein et al., 1994; Marley and Gallager, 1989; Miller et al., 1988a, 1989; Schoch et al., 1993; Wong et al., 1994; Wu et al., 1994). Regional differences have been demonstrated, with down-regulation of benzodiazepine-binding sites most likely to be found in cortex, hippocampus, and amygdala. The $GABA_A$ receptor is the predominant site of the neurochemical action of this class of compounds (Schoch et al., 1985), although some benzodiazepines also bind to the peripheral-type benzodiazepine site present in the central nervous system (CNS), as well as in other tissues (Kreuger, 1991; Miller et al., 1988b; Weizman and Gavish, 1989).

We have previously described a model of lorazepam tolerance which produces behavioral changes consistent with prior studies and changes in binding and function at the $GABA_A$ receptor (Miller et al., 1988a). In that study, plasma and brain lorazepam concentrations were proportional to dose and were constant over time, indicating that the tolerance was

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not pharmacokinetic. This is consistent with most other studies, which have shown that the development of tolerance does not correlate with decreased benzodiazepine levels in blood plasma, cerebrospinal fluid, or brain tissue (Gonsalves and Gallager, 1988; Greenblatt and Shader, 1986; Haigh et al., 1986; Lister et al., 1983; Loscher and Schwark, 1985; Tyma et al., 1984). In addition, benzodiazepine receptor occupancy in vivo was highly correlated with rotorod ataxia during both the first 1 to 2 days after exposure and at 7 to 14 days after exposure. Changes in benzodiazepine receptor binding in vitro and chloride uptake, however, were not assessed for the full 14-day course of treatment. At present, few studies have been conducted which correlate CNS changes with behavioral tolerance to draw any firm conclusions regarding a neural mechanism for this phenomenon.

In recent years, five different GABA receptor subunit families have been identified, most of them having multiple subtypes. The $GABA_A$ receptor complex is thought to be a heteropentameric glycoprotein composed of combinations of these multiple polypeptide subunits. Different affinities of benzodiazepines for the $GABA_A$ receptor have been described both in vivo and in recombinant-expressed receptors composed of different subunit combinations (Doble and Martin, 1992; Dubnick et al., 1983; Heninger et al., 1990; Leonard, 1993; Lüddens and Wisden, 1991; Primus and Gallager, 1992; Pritchett et al., 1989). Evidence from in situ hybridization studies has also accumulated to suggest that $GABA_A$ receptor complexes with different subunit compositions may be differentially located in the CNS and this may explain the heterogeneity of behavioral responses to different benzodiazepine ligands (Davies et al., 1994; Doble and Martin, 1992; Dubnick et al., 1983; Klein et al., 1995; Pesold et al., 1997). Autoradiographic studies can now be used to determine whether differences in the affinity of benzodiazepines for this receptor exist in different brain areas. To date, there have been few receptor autoradiographic studies and fewer still that correlate these changes with pharmacodynamic tolerance (Brett and Pratt, 1995; Tietz et al., 1986). In the present study, we examined open-field activity, benzodiazepine binding in vitro, overall function of the $GABA_A$ receptor complex as indicated by chloride uptake, and [^{125}I]diazepam receptor autoradiography at Days 1 and 14 of a course of lorazepam previously demonstrated to produce behavioral tolerance and receptor down-regulation (Miller et al., 1988a). Our intent is to correlate specific regional changes in binding with pharmacodynamic tolerance in the same animal. In addition, in vitro binding and chloride uptake data following 14-day lorazepam exposure, not previously described, were examined.

2. Methods

2.1. Materials

Male Crl: CD-1(ICR)BR mice, 6 to 8 weeks of age, were purchased from Charles River Laboratories (Wilmington, MA), maintained on a 12-h light/dark cycle and given food and water ad libitum. [^{3}H]flunitrazepam (specific activity 71 Ci/mmol) and [^{3}H]flumazenil (specific activity 81 Ci/mmol) were purchased from New England Nuclear (Boston, MA). Lorazepam was generously donated by its pharmaceutical manufacturer. All other reagents were obtained from standard commercial sources.

2.2. Drug administration

Lorazepam was dissolved in polyethylene glycol (PEG) 400 and loaded into Alzet osmotic pumps (Alza, Palo Alto, CA). Under brief ether anesthesia, the pumps were implanted subcutaneously. Drug delivery was at a rate of 2 mg/kg/day for 1 or 14 days. The dose of lorazepam was chosen based on prior studies (Galpern et al., 1990). To control for unexpected, but possible, PEG 400 effects, a subset of control mice received PEG 400 alone (vehicle-treated). A second group of control animals in the autoradiography study did not undergo pump implantation. Following sacrifice, brains were removed. The right hemisphere was processed for membrane binding and the left hemisphere was processed for cryotomy.

2.3. Open-field activity

Activity for all groups, including distance traveled, rears, and stereotypy, was assessed in 5-min intervals for 20 min in an Omnitech Digiscan apparatus (Columbus, OH) on Days 1 and 14 after the pumps were implanted. Between each run, the interior of the activity chamber was cleaned with 70% ethanol and dried. All testing occurred between 9:00 a.m. and 12:00 noon. Averages of each parameter for the total 20-min test period were computed.

2.4. Lorazepam concentrations

Trunk blood was obtained after sacrifice and plasma was separated and frozen at -20°C until analysis. Cortical tissue was obtained after sacrifice and homogenized in 1 ml of double-distilled water with a Polytron (setting 7, 15 s). Homogenates were frozen at -20°C until analysis. Lorazepam concentrations were determined by electron-capture gas chromatography according to previously determined methods (Greenblatt et al., 1978).

2.5. [^{3}H]flunitrazepam binding

Benzodiazepine binding in vitro was performed in mouse cortical synaptosomes (P_2) as previously described using [^{3}H]flunitrazepam (Miller et al., 1988a). Briefly, samples (about 50 μg protein) were incubated with [^{3}H]flunitrazepam (0.1–10 nM) in the presence (total binding) or absence (nonspecific binding) of flumazemil (10 μM) for 45 min at 4°C. Tris–HCl buffer is added to all tubes to achieve a final volume of 0.5 ml. Incubations were terminated by filtration

and filters were subsequently washed and counted. Standard analyses of binding data were used to determine the number of binding sites (B_{max}) and the apparent affinity (K_d).

2.6. Muscimol-stimulated [^{36}Cl] uptake

Chloride uptake was performed using cortical synaptoneurosome preparations as previously described (Miller et al., 1988a). Briefly, cortical synaptoneurosomes were prepared and resuspended in assay buffer (145 mM NaCl, 5 mM KCl, 1 mM MgCl, 1 mM $CaCl_2$, 10 mM HEPES, pH 7.4). After incubation for 10 min at 30°C, 100 μl of membrane suspension was mixed with 100 μl of a solution containing muscimol (0.003–100 μM) and $^{36}Cl^-$ (0.2 μCi/ml assay buffer). After 6 s, the incubation was terminated by the addition of 0.5 ml cold assay buffer containing 6 μM picrotoxin and filtration on Whatman GF/C filters by a Brandel M24 apparatus. Filters were washed twice with cold buffer and counted by scintillation spectrometry.

2.7. Tissue preparation

Brains from male CD-1 mice were rapidly removed on ice and rinsed with ice-cold saline. Brains were then blocked 0.5 mm perpendicular to the midsagittal line, frozen in *N*-hexane cooled to −80°C with dry ice, sealed in aluminum foil, and stored at −30°C until sectioning. Frozen and blocked rat brains were sectioned at 12 μm thickness. Prior to tissue sectioning, microscope slides were first cleaned in a 0.2 N NaOH solution in diethylpyrocarbonate-treated water (to maintain an RNase-free surface) and dried in 100% ethanol. Clean slides were dipped for 20 s in poly-D-lysine and dried overnight at 40°C prior to use. Care was taken to handle all slides with gloves to avoid possible RNase contamination. Brains were sectioned along the coronal plane. Three adjacent sections were adhered per slide so that values from individual animals for each radioligand studied were replicated. Once sections were adhered to slides, they were allowed to air dry and then were stored in a dessicator at −80°C until use. Sections were taken at or about the level equivalent to 2.4 mm posterior to bregma in the rat brain. Neuroanatomical features based on standard stereotaxic coordinates were used as landmarks to align brains during sectioning. Occasional sections were collected on separate noncoated slides and rapidly stained with 0.5% toluidine blue in ethanol confirmed that brain alignment was parallel to the coronal plane.

2.8. [^{125}I]diazepam receptor binding

Slides were removed from storage and allowed to warm to room temperature for 15 min. Slides were preincubated in phosphate-buffered sucrose (0.32 M sucrose, 0.2 M Na_2HPO_4, 0.2 M NaH_2PO_4, pH 7.4) for 30 min at 24°C. Phosphate-buffered sucrose containing 10 nM [^{125}I]diazepam (200 μl) was pipetted onto tissue sections and a 20 × 40-mm coverslip was floated onto the solutions, which assured even coverage of tissue sections and minimizes evaporation. Slides were incubated for 60 min at 4°C, rinsed in PBS (3 × 1 min), rinsed in distilled water (three dips), and dried under a cool stream of air.

2.9. Quantitative analysis of autoradiograms

Following incubation with the radioligand, sections were apposed to Kodak SB-5 single-emulsion X-ray film. Exposure times varied somewhat for radioligands but were approximately 1–2 weeks for [^{125}I]-labeled compounds. We have observed that single-emulsion films yield lower background density and, therefore, better signal-to-noise ratio than conventional double-emulsion X-ray films. Autoradiograms were processed using Kodak GB-X developer and fixer, rinsed for 12 min in running tap water and allowed to air dry. Tissue sections were then stained with Azure B to afford visualization of brain section morphology. Appropriate commercial radioactive standards (American Radiolabeled Chemicals, St. Louis, MO) were apposed to film simultaneously so that densities from tissue sections can be converted to specific activity (nanocuries) per gram of protein. Image analysis was performed using Image Analyst software (Acuity, Billerica, MA). This software package runs on a Macintosh IIfx computer using a Pulnix TM-7 CCD video camera and a Perceptics Pixel Buffer frame grabber. Images of both stained tissue sections as well as autoradiographs of receptor binding are aligned and digitized. The stained tissue sections are then used to create field-delimiting masks in order to isolate and quantitate autoradiographic densities from specific areas of the brain.

2.10. Data analysis

Film densities are calibrated using the densities from [^{14}C] standards and an estimate of femtomoles per gram of protein can be computed based on the known specific activity of the labeled probe (Miller, 1991). Data from binding studies were analyzed using the RADLIG program (version 4.0). Comparisons between groups were performed using analysis of variance (ANOVA) with Dunnett's post hoc test.

3. Results

3.1. Open-field activity

A significant decrease in total distance traveled [$F(1,6) = 116.284$, $P < .0001$], total number of rears [$F(1,6) = 103.532$, $P < .0001$], and total stereotypy [$F(1,6) = 38.599$, $P < .001$] was observed on Day 1 of lorazepam treatment. By Day 14, there was no difference

between lorazepam- and vehicle-treated animals on any of the three behavioral parameters examined (Fig. 1).

3.2. Lorazepam concentrations

The average brain concentrations were 583 and 478 ng/g, respectively, at Days 1 and 14 (using an average brain weight of 475 mg). The average plasma concentrations were 710 and 606 ng/ml, respectively, at Days 1 and 14. Furthermore, the average ratios of brain to plasma concentrations remained constant over time (0.82 and 0.79, respectively). Thus, continuous administration of lorazepam by osmotic pumps was accurate, with no significant change over the 14-day period. It must be noted, however, that the brain levels in the present study are 3–4 times higher than those previously reported, while plasma levels are 9–10 times higher (Miller et al., 1988a). This discrepancy could be due to an error in dosage or analytical procedure as well as osmotic pump malfunction.

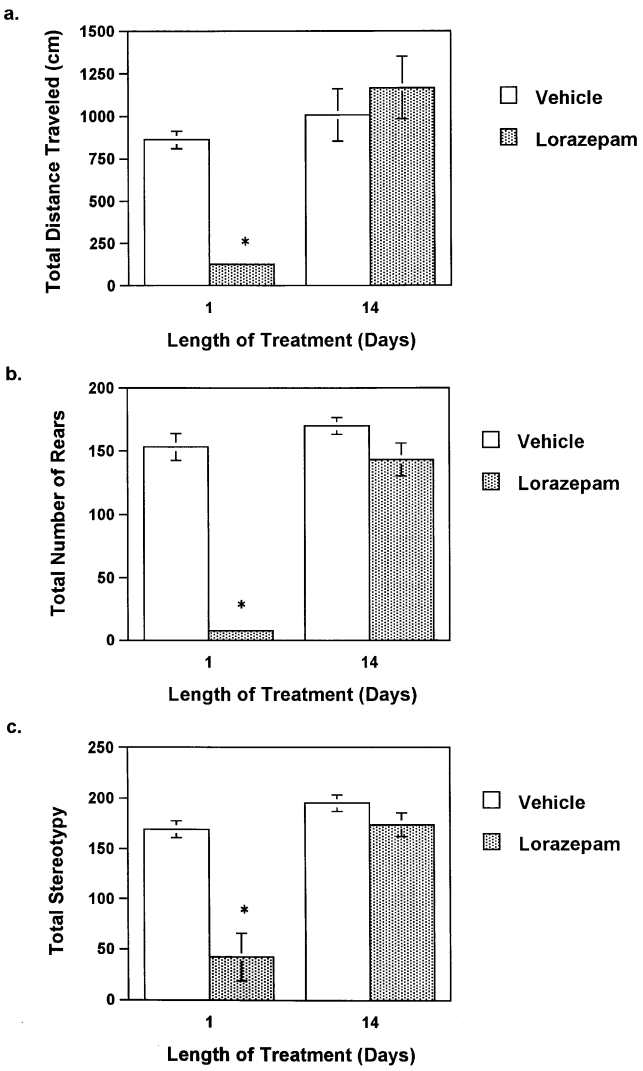

Fig. 1. Effect of chronic lorazepam administration on open-field activity. Data from (a) distance traveled, (b) number of rears, and (c) number of stereotypies were recorded at 5-min intervals for a total of 20 min. Lorazepam (2 mg/kg/day) or vehicle was chronically administered via osmotic pump for 1 or 14 days ($n \geq 3$). Averages for each parameter were computed for the entire test period. Bars represent mean response ± S.E.M. Error bars for lorazepam Day 1 in (a) and (b) have values that are too small to be depicted relative to y-axis scale. Significant differences from vehicle are indicated by * ($P < .05$) as determined by ANOVA and Dunnett's post hoc test.

Table 1
Benzodiazepine receptor binding in cortex following chronic lorazepam administration

	Day 1		Day 14	
Treatment	K_d (nM)	B_{max} (pmol/mg protein)	K_d (nM)	B_{max} (pmol/mg protein)
Vehicle	1.55 ± (0.14)	3.71 ± (0.75)	1.49 ± (0.11)	2.84 ± (0.35)
Lorazepam	1.57 ± (0.30)	4.37 ± (1.39)	1.47 ± (0.10)	2.47 ± (0.27)*

Benzodiazepine binding ($n \geq 4$) was performed in cortical synaptosomal membranes (P_2) using [^{3}H]flunitrazepam. Data represent mean values ± S.E.M.

* Significant differences ($P < .05$) from Day 1 as determined by ANOVA and Dunnett's post hoc test.

3.3. Benzodiazepine binding in vitro

There was no difference in K_d (4%) on Day 14 of lorazepam treatment when compared to Day 1 (Table 1). A similar comparison demonstrated a significant decrease (40%) in B_{max}, however, on Day 14 in the lorazepam treatment group [$F(1,19) = 5.169$, $P < .05$]. There were no significant differences in K_d (7%) or B_{max} (20%) in the vehicle-treated animals.

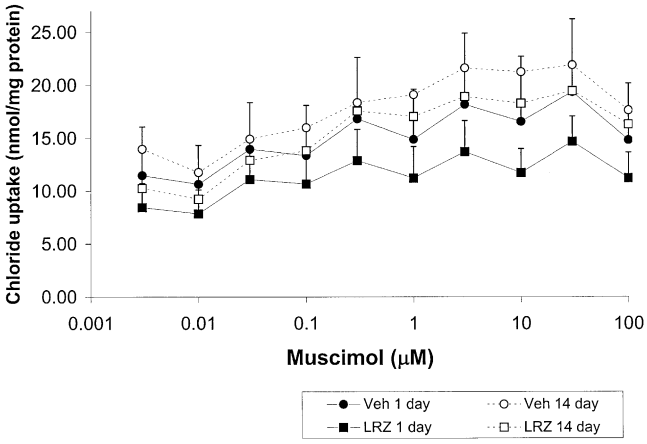

Fig. 2. [$^{36}Cl^-$] uptake in synaptoneurosomes during chronic lorazepam administration. Lorazepam (2 mg/kg/day) or vehicle was chronically administered via osmotic pump for 1 or 14 days ($n \geq 4$). Synaptoneurosomes were prepared from the cortex and incubated for 6 s with [$^{36}Cl^-$] and varying concentrations of muscimol. Results are from a typical experiment performed in triplicate and repeated twice. There were no significant differences between the groups.

3.4. Muscimol-stimulated [^{36}Cl] uptake

There were no differences in chloride uptake between vehicle- and lorazepam-treated animals on Day 1 or Day 14 (Fig. 2).

3.5. [^{125}I]diazepam receptor binding

Receptor binding on Day 14 of lorazepam treatment was assessed in cortical layers 2–6 as well as areas CA1, CA3, and the dentate gyrus (Fig. 3). Significant decreases in receptor binding were observed in cortical layers 5 [$F(1,9)=8.55$, $P<.05$] and 6 [$F(1,9)=5.112$, $P<.05$] in the lorazepam-treated animals compared to untreated and vehicle-treated animals. Binding in the vehicle-treated animals was also diminished compared to untreated controls, but the differences were not significant. This is most likely due to the large variability in the control and vehicle groups. No significant differences among the groups were seen in the hippocampus or dentate gyrus, although a similar trend towards decreased [^{125}I]diazepam binding was seen in CA1. Representative autoradiograms of [^{125}I]diazepam binding are found in Fig. 4.

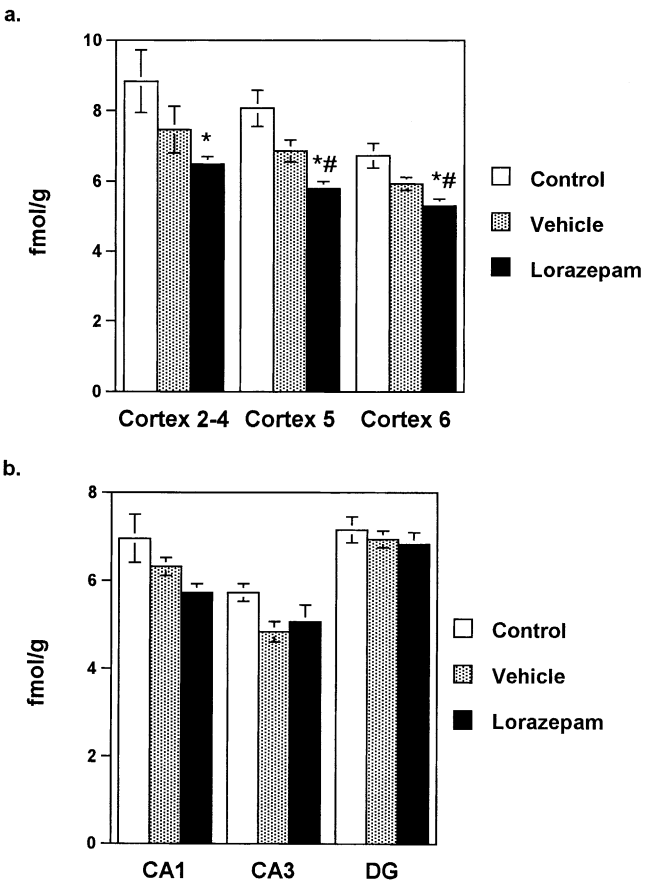

Fig. 3. Effect of chronic lorazepam administration on [^{125}I]diazepam receptor binding. Lorazepam (2 mg/kg/day) or vehicle was chronically administered via osmotic pump for 14 days ($n \geq 5$). Control animals did not have an osmotic pump implanted subcutaneously. [^{125}I]diazepam receptor binding was performed on tissue sections from (a) layers II–VI of the cerebral cortex and (b) the dentate gyrus (DG) and areas CA1 and CA3 of the hippocampus. Bars represent mean [^{125}I]diazepam binding (fmol/g) ± S.E.M. Significant differences from control and vehicle are indicated by * ($P<.05$) and $^{\#}$ ($P<.05$), respectively, as determined by ANOVA and Dunnett's post hoc test.

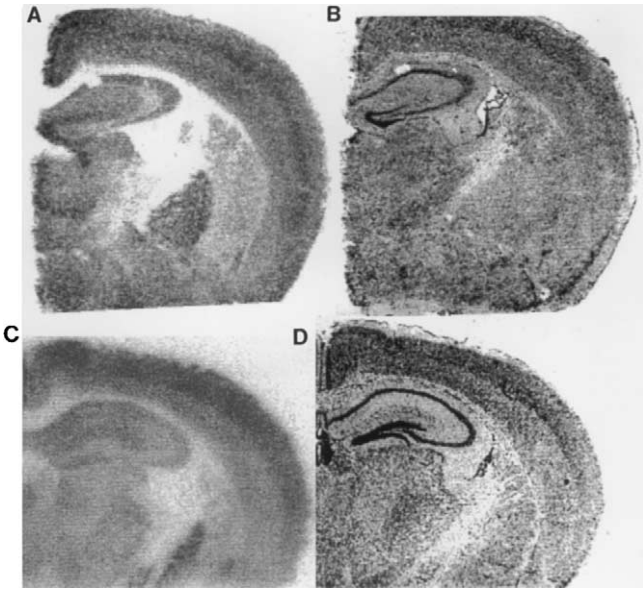

Fig. 4. Representative autoradiograms of [^{125}I]diazepam binding following chronic lorazepam administration. Lorazepam (2 mg/kg/day) or vehicle was chronically administered via osmotic pump for 14 days ($n \geq 5$). Control animals did not have an osmotic pump implanted subcutaneously. Cross sections represent (A) an autoradiogram of a 14-day lorazepam-treated animal, (B) the corresponding Azure B-stained slide of the 14-day lorazepam-treated animal in (A), (C) an autoradiogram of a 14-day control animal, (D) corresponding Azure B-stained slide of the 14-day control animal in (C). Note the decreased cortical and hippocampal binding in the lorazepam-treated animals.

4. Discussion

The present study represents an analysis of behavioral and neurochemical parameters associated with tolerance. We have previously described a model of tolerance at this dose of lorazepam that produces consistent changes in behavior as well as binding and function at the $GABA_A$ receptor (Miller et al., 1988a). It was thus possible to examine the close temporal association between decreased receptor binding in several brain regions and the development of tolerance to behavioral effects in this study. The use of implanted pumps to allow continuous delivery of drugs avoids possible limitations inherent in intermittent injection. This was confirmed by data from the present study, which demonstrated that brain and plasma levels remained constant over the 14-day period. Open-field behavior was evaluated as a pharmacodynamic parameter due to its non-invasive nature, simplicity of measurement, and our prior experience using this technique as an indicator of both

benzodiazepine effects and tolerance (Miller et al., 1988a, 1989, 1990).

In vitro binding results indicate that chronic exposure to lorazepam results in down-regulation of the benzodiazepine receptor in the cortex. Receptor down-regulation involves a decrease in the number of sites (B_{max}) rather than a decrease in apparent affinity (K_d). A decrease in B_{max} but not in K_d on Day 14 of lorazepam treatment in the present study is consistent with much of the prior work on chronic benzodiazepines (Allan et al., 1992; Byrnes et al., 1993; Miller et al., 1988a, 1989; Wu et al., 1994). The observed decrease in cortical specific binding was most likely due to a decrease in receptor number rather than in apparent affinity. Given these data, it is unlikely that a change in the affinity of the benzodiazepine-binding site is a potential neural mechanism for the development of tolerance.

The in vitro benzodiazepine receptor binding results correlate well with experiments using the same model and autoradiographic techniques. The two main differences between autoradiographic techniques and in vitro binding using membranes lie mainly in the tissue preparation and quantification procedures. The agreement of the present results with those obtained with membrane binding techniques confirm that, despite the different procedures, both techniques are comparable. However, autoradiographic studies, while technically more difficult, offer the advantage of greater anatomical accuracy. Soria et al. (1995) have recently conducted saturation kinetic studies of benzodiazepine-binding sites in a number of brain areas from the mesencephalon, cortex, hippocampus, and cerebellum and have determined that the values for K_d and B_{max} obtained with autoradiography correlate well with those of membrane binding.

A decrease in benzodiazepine binding was seen in cortical layers 5 and 6 following 14-day lorazepam treatment using autoradiographic techniques when compared to vehicle-treated animals. This is consistent with previous in vivo and in vitro benzodiazepine receptor binding done in this laboratory and others (Allan et al., 1992; Byrnes et al., 1993; Miller et al., 1988a, 1989; Wu et al., 1994). It is likely that this decrease is due to a reduction in receptor number based on the in vitro binding results of this study as well as other reports in which down-regulation of benzodiazepine-binding sites was found in the cortex (Allan et al., 1992; Byrnes et al., 1993; Miller et al., 1988a, 1989; Wu et al., 1994). In addition, the majority of studies that have measured benzodiazepine affinity, including the present one, have demonstrated that chronic benzodiazepine treatment does not alter affinity (Allan et al., 1992; Braestrup et al., 1979; Miller et al., 1988a, 1989; Rosenberg and Chiu, 1981). As noted earlier, there have been few studies of chronic benzodiazepine administration using receptor autoradiography. The present results are consistent with Tietz et al. (1986) who showed similar receptor down-regulation after chronic oral administration of flurazepam in rats. Brett and Pratt (1995) recently demonstrated changes in the nucleus accumbens following chronic diazepam treatment. Less marked effects were noted in the lateral habenula, dorsal raphe, and substantia nigra pars compacta. However, cerebral cortex was not examined in this study.

Tolerance to behavioral effects was also observed on Day 14 of lorazepam administration. Much of the prior work on tolerance to general locomotor activity effects has demonstrated a sedative effect of chronic benzodiazepine administration as evidenced by decreased activity (Goldberg et al., 1967; Matsubara and Matsushita, 1982; Miller et al., 1988a, 1989, 1990). The present results demonstrate that benzodiazepine receptor down-regulation in the cortex is associated with behavioral tolerance to benzodiazepines. It must be noted, however, that this association may not apply to different benzodiazepines or different behavioral tests. The temporal association between pharmacodynamic effects and receptor down-regulation in the present study is consistent with results obtained from prior acute and chronic dosage studies in our laboratory and others. These observed changes in binding, in turn, may not be associated with changes in receptor function. No significant differences in muscimol-stimulated chloride uptake in cerebral cortex were found between vehicle- and lorazepam-treated animals on Day 1 or Day 14 in this study. While previous work from our laboratory and others has demonstrated a decrease in chloride uptake following chronic lorazepam administration for 7 days (Galpern et al., 1990; Marley and Gallager, 1989; Miller et al., 1988a), chloride uptake has not been measured after 14 days of benzodiazepine infusion. Differences between the present study and previous work from our laboratory may be due to the increased brain lorazepam levels demonstrated in this study (see Results).

These results indicate that tolerance to benzodiazepines is complete following 2-week lorazepam treatment using both pharmacodynamic and receptor binding techniques. This regimen may be a possible model that can be applied to pharmacologic interventions to prevent or reduce tolerance. Tolerance to lorazepam may involve not only the benzodiazepine-binding site, but also overall function at the $GABA_A$ receptor. It is presently uncertain whether this down-regulation involves the entire $GABA_A$ receptor complex. Studies that have addressed effects of chronic treatment with benzodiazepines report conflicting results. Prior studies in our laboratory have demonstrated a decrease in chloride channel binding in tolerant mice following 1 week of lorazepam or alprazolam treatment (Miller et al., 1988a, 1989), which was not seen after the 14-day lorazepam treatment in this study. Other studies from this laboratory as well as other investigators have shown that repeated benzodiazepine administration does not lead to a change in the number of chloride channels (Galpern et al., 1991; Heninger and Gallager, 1988). It is also possible that chronic administration alters receptor conformation that could modify coupling between the chloride channel and GABA. It has previously been demonstrated that chronic exposure to benzodiazepines decreases the coupling of allosteric sites

and is, therefore, a putative neural mechanism for benzodiazepine tolerance (Allan et al., 1992; Brett and Pratt, 1995; Heninger and Gallager, 1988; Hu and Ticku, 1994; Klein et al., 1994; Tietz et al., 1986; Wong et al., 1994). This would account for the alterations in benzodiazepine receptors observed in the present study.

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