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Dorsal Raphe and Substantia Nigra Response to Flumazenil in Diazepam-Dependent Rats

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WALA, E. P., J. W. SLOAN AND X. JING. *Dorsal raphe and substantia nigra response to flumazenil in diazepam-dependent rats.* PHARMACOL BIOCHEM BEHAV **58**(1) 221–229, 1997.—Flumazenil (FLU; 25 μg) and DMSO–vehicle were focally injected (1 μl) into the substantia nigra (SN) and the dorsal raphe nucleus (DR) in rats chronically implanted with silastic capsules containing diazepam (DZ; 540 mg/week). FLU precipitated an abstinence syndrome in the SN as indicated by a significant abstinence score, several abstinence signs and reduced total power of the fast frequency bands of the electroencephalogram (EEG) in the injections sites frontal cortex, (FC) and hippocampus (H). In contrast, FLU did not produce an abstinence syndrome in the DR, and its effect on the power of the EEG in DR, FC and H was not significantly different from that of the DMSO–vehicle. The data show regional heterogeneity in the response of the SN and the DR to chronic DZ treatment in terms of a focally precipitated abstinence syndrome. © 1997 Elsevier Science Inc.

Diazepam dependence Precipitated abstinence Flumazenil Substantia nigra Dorsal raphe Focal injection

CHRONIC exposure of rats to diazepam (DZ) slowly released from subcutaneously implanted silastic capsules results in physical dependence as indicated by an abstinence syndrome precipitated by the systemic administration of the central benzodiazepine receptor antagonist, flumazenil (FLU) (31,62). The diversity of FLU-evoked abstinence signs and symptoms suggests that the dependence-producing properties of DZ vary from one brain site to the other.

There is a line of evidence for regional brain heterogeneity in response to chronic benzodiazepine (BZ) treatment as indicated by electrophysiological, biochemical and behavioral studies. The existence of multiple $GABA_A$ subunits and their heterogenous distribution in the brain suggests that the $GABA_A$/BZ complex is regulated independently in different brain structures and that the GABA receptor shows a regional difference to chronic occupation of the BZ-recognition site(s) [for review, see (18)].

The dorsal raphe nucleus (DR) and the substantia nigra pars reticulata (SN) are interesting brain loci for the study of physical dependence on and abstinence from BZs. These two brain sites receive a rich GABAergic innervation; however, reticulata neurons are inhibited tonically by GABA input but a lack of tonic GABAergic input in serotonergic DR neurons. In rats, neurons of the DR nucleus and reticulata neurons of the SN are affected differently by long-period exposure to DZ in terms of their sensitivity to iontophoretically applied GABA and their responsiveness to additionally administered BZ agonist and antagonist [for reviews, see (13,14,17–19,46)].

Chronic administration of BZs in rats results in a regional variation in the degree and rate of downregulation of BZ binding sites, which is significantly greater in the SN than in other brain areas (49). The SN shows a higher degree of labeling of BZ-1 type receptors than the DR (35) and different combinations of GABA subunits in the SN pars reticulata (α_1, $\beta_{2,3}$, γ_2) and the DR (α_1, α_3, $\beta_{2,3}$, γ_2, and α_3, γ_2) (10). The BZ-1 recognition site shows specificity for the α_1 subunit, whereas non-BZ-1 site(s) are associated with the α_2 and α_3 subunits (36). Chronic DZ treatment produces regionally specific decreases in levels of mRNA in the α_1 subunit of the $GABA_A$ receptor (23). However, the relationship between the mRNA decrease in the α_1 subunit and downregulation of BZ-1 receptors has not been established [for references, see (66)].

Decreased GABAergic function and a disruption of the $GABA_A$/BZ coupling may describe the tolerance to some pharmacological actions of BZs (15,41,47). The baculovirus Sf9 expression system has been used to show that coupling at

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individual $GABA_A$ subtypes decreases after long-term BZ exposure and that the uncoupling, which replicates changes after chronic BZ treatment in vivo, can be reversed by a brief exposure to FLU, which suggests that alteration in the GABA/BZ receptor complex due to chronic BZ treatment is mediated directly at the GABA receptor (40).

It is not known if the same mechanism is responsible for the development of tolerance to some effects of BZs and for production of physical dependence on BZs. However, rats that become tolerant to the anxiolytic effects of BZs (plus-maze test) manifest an anxiogenic response during withdrawal from the chronic BZ treatment, whereas rats that fail to develop tolerance to the anxiolytic effects do not show an anxiogenic response on withdrawal (2,9). It is interesting, therefore, to determine if the withdrawal syndromes evoked from the SN, where chronic DZ treatment does not affect GABAergic function (60,63), and from the DR, where chronic exposure to DZ results in GABA subsensitivity (15,16), are quantitatively and qualitatively different. In the present study, we have determined the ability of focally microinjected FLU to precipitate an abstinence syndrome and to change the brain electrical activity in the SN pars reticulata and the DR nucleus in rats exposed to DZ slowly released from silastic capsules.

METHODS

Animals

The studies were conducted in female Sprague-Dawley rats (250 g) in which the estrus cycle was not controlled. The rats were housed in accordance with the Declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals. The protocol of the experiments was approved by the Institutional Animal Care and Use Committee of the University of Kentucky. All surgical procedures were performed under sterile conditions and ketamine anesthesia (80 mg/kg, IP). The rats were killed with pentobarbital (120 mg/kg, IP).

Implantation of Guide Cannulae and Electroencephalogram (EEG) Electrodes

The rats were mounted in a Kopf stereotaxic instrument, and their brains were given implants at the interaural coordinates (38) with an indwelling guide cannula-recording electrode (G-22, insulated except at the tip with epoxylite 6001M) directed toward the SN (AP = 3.7 mm, LL = 2.4 mm, V = 5 mm) or the DR (AP = 1.2 mm, L = 0 mm, V = 6 mm). Intracranical (E363/1) stainless steel electrodes (Plastics One) were implanted into the hippocampus (H; AP = 3.7 mm, LL = 3 mm, V = 6 mm) and frontal cortex (FC; AP = 10.2 mm, LL = 2 mm, V = 8.2 mm). An indifferent electrode was imbedded in the skull behind lambda. The electrodes were connected to a pedestal and secured to the bone with acrylic cement.

Chronic Administration of DZ

After recovery from surgery (7–10 days), the rats were chronically exposed to DZ according to the previously described procedure (16), with minor modifications (31). Three silastic capsules (11 cm long, Medical Grade silastic tubing; inner diameter of 0.147 cm × outer diameter of 0.195 cm; sealed at both ends with silicone type-A medical adhesive) filled with 180 mg DZ were implanted subcutaneously at weekly intervals. Previously implanted capsules were not removed throughout the duration of the chronic studies. The control rats were implanted with empty silastic capsules.

Precipitation of Abstinence

After 3 weeks of exposure to the implanted capsules, the rats were placed in an observation chamber (a large round Faraday cage with a grounded solid metal wall) and connected to a Grass 78D EEG/polygraph through a commutator (SL6C) and concentric mercury swivel (Plastics One). The EEG and signs and symptoms of precipitated abstinence were recorded simultaneously for 10 min prior to (baseline) and 40 min after focal injections. The abstinence signs were recorded in 5-min epochs on the standardized observation forms and on the EEG tracing.

One microliter of FLU (25 mg/ml in DMSO) was injected through the chemotrode (G-28) into the SN (V = 1.8 mm) or the DR (V = 3.6 mm). All injections were made without handling the rat by using a Hamilton syringe connected to the chemotrode with polyethylene tubing (PE-20). The tightness of the fit between the guide cannula and the chemotrode minimized the reflux of injected solution. Each rat served as its own control with respect to the effect produced by the DMSO–vehicle (1 μl) injected into the SN or the DR 3 days later. A dose of FLU was selected from the pilot study, which showed that 25 μg of FLU precipitated a well-defined abstinence syndrome in several brain loci. The concentration of injected solutions was limited by FLU solubility in DMSO.

Abstinence Signs

Several scored signs of abstinence such as convulsive phenomena (clonic and tonic–clonic convulsions, twitches and jerks), motor manifestation of withdrawal (head and body tremors, jumping, turning, backing), motor dyskinesia (writhing) and affective aberration (vocalization, arched back) were counted, recorded and summed for each 5-min observation period to generate the time action course of the abstinence score. The other unscored abstinence signs (stretching, chewing, ear twitching, digging, head bobbing, hot foot walking, blinking, poker tail, scratching, rearing, wet dog shakes, ataxia) and some items related to activity (sitting, walking, exploring, standing, prone, curled) were also recorded and summed for each 5-min epoch but were not included in total abstinence score. The respiration rate was counted once for each epoch (31).

To normalize the data for differences in baseline values for pre-FLU and pre-DMSO observations, the score for each sign of abstinence was analyzed for each rat at each postinjection observation period as follows. To generate normalized FLU and DMSO scores, the mean control value (pre-FLU or pre-DMSO baseline) was subtracted from each of the eight 5-min post-FLU or post-DMSO scores, respectively. The time action curves for normalized individual abstinence signs were obtained by subtracting the normalized DMSO score from the normalized FLU score [(FLU − pre-FLU) − (DMSO − pre-DMSO)].

The data are presented as the time action curve of the abstinence score generated before and after focal injections of FLU and DMSO. In addition, the abstinence score and scores for individual abstinence signs are presented as the mean areas under the time action curves (0–40 min) for normalized scores ± SEM for *n* rats in a group.

Brain Electrical Activity

The EEG was recorded from the sites of focal injections (SN or DR) and from the H and FC for 10 min prior to (baseline) and 40 min after focal injections of FLU and DMSO.

Each rat served as its own control with respect to preinjection baseline. The EEG signals were sampled at 256 samples/s by using a data acquisition board and were stored for offline analysis. The epochs containing artifacts were discarded after visual inspection of the polygraph records. Fast Fourier transform was performed at 4-s epochs after filtering signals with the bandpass filter (1–32 Hz). The power spectrum was averaged over 5-min intervals, and the total distribution across frequency ranges was determined. The frequencies were grouped into five traditional bands: 1–4 (delta), 4–12 (theta and alpha), 12–18 ($beta_1$), 18–26 ($beta_2$) and 26–32 Hz (fast beta). To normalize data for between-subjects differences in control EEG, relative changes in total power (1–32 Hz) and in power for the different frequency bands were calculated for each rat with respect to its own pre-FLU and pre-DMSO baseline, respectively. The data are presented as the mean ± SEM percentage of the increment or decrement over the individual baseline. The time course of relative changes in power of the EEG and the time course of precipitated abstinence scores were for the same *n* rats in each group.

Plasma Levels of DZ and its Metabolites

Blood was collected in weekly intervals by venipuncture of the tail vein. Plasma levels of DZ and its metabolites nordiazepam (ND), oxazepam (OX), 4-hydroxydiazepam (OH-DZ) and temazepam (TM) were determined by HPLC, as previously described (31).

Histology

After completion of the experiments, the locations of the chemotrode and the EEG electrodes were identified. After perfusion with formalin (while under deep pentobarbital anesthesia), the brains were removed and fixed in formalin. Frozen sections were cut at 32 μ, mounted on gelatin-coated slides, stained with neutral red and examined under the light microscope. Data from the rats with accurately placed guide cannulae and electrodes are presented.

Statistics

One-way analysis of variance for repeated measures and post hoc *t*-test were used for statistical analysis of the data ($p < 0.05$ was considered significant).

RESULTS

Female rats chronically exposed to DZ slowly released from silastic capsules (540 mg/week) did not show overt signs of sedation or ataxia. The average increase in body weight was equal to 2.6 ± 4.18 g. Implantation of guide cannulae and EEG electrodes did not produce motor impairment.

After 3 weeks of exposure to DZ, the mean steady-state plasma levels (μg/ml) of DZ and its metabolites were: DZ = 2.22 ± 0.55, ND = 0.56 ± 0.10, OX = 0.26 ± 0.12, OH-DZ = 0.52 ± 0.33 and TM = 0.45 ± 0.11.

Figure 1 illustrates the time course of abstinence scores produced by the focal administration of FLU or DMSO–vehicle into the DR (Fig. 1A) and into the SN (Fig. 1B) in rats chronically treated with DZ and into the SN (Fig. 1C) in control rats. The data indicate that in rats exposed to DZ the focal injection of FLU (25 μg) into the DR does not produce abstinence. In contrast, in rats undergoing identical chronic treatment with DZ, focal injection of FLU (25 μg) into the SN precipitated a significant abstinence syndrome within 5 min. In control rats, similar injection of FLU into the SN did not produce abstinence. Analysis of variance indicates significant between-treatments differences in FLU-induced abstinence scores in the SN [$F(2,8) = 38.7$, $p < 0.0001$] but not in the DR [$F(2,8) = 4.09$, NS].

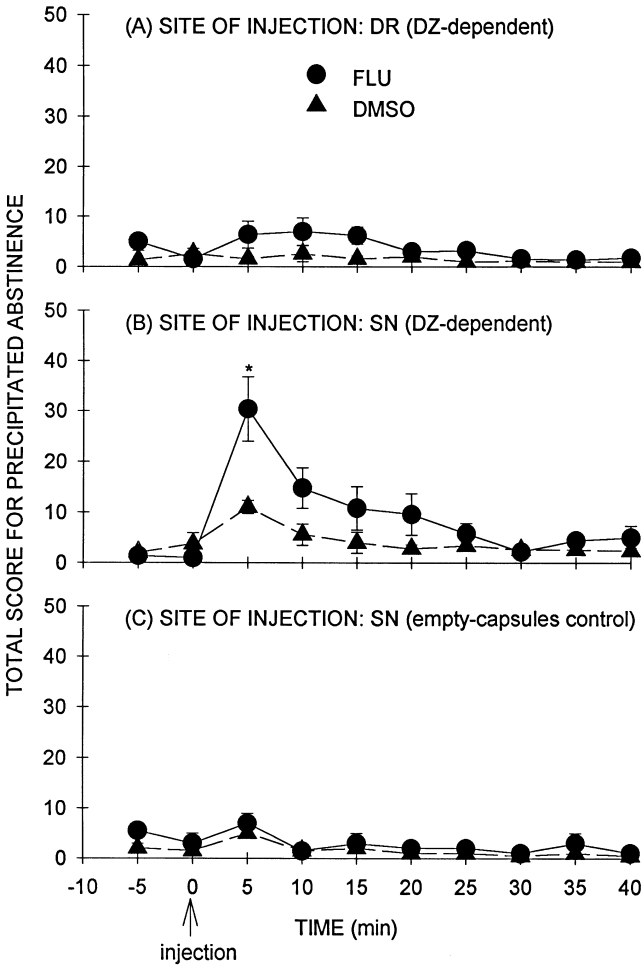

FIG. 1. The time course of the abstinence score produced by microinjections (1μl) of flumazenil (FL; 25μg) and DMSO–vehicle into the dorsal raphe nucleus (DR; A) and the substantia nigra pars reticulata (SN; B) in rats chronically exposed to diazepam (DZ) slowly released from silastic capsules (540 mg/week) and into the SN (C) in rats chronically implanted with empty capsules. Data are the mean ± SEM of 5 rats in each group. Asterisk indicates significant difference from DMSO–vehicle ($p < 0.05$, *t*-paired test).

Table 1 summarizes the total scores for abstinence and scores for some individual abstinence signs produced by focally administered FLU in the DR and in the SN. The scores were calculated as areas under the time action (0–40 min) curves. As can be seen, FLU does not evoke convulsive signs of abstinence (clonic, tonic–clonic convulsions, twitches and jerks) either in the DR or in the SN. However, FLU precipitates tachypnea, head and body tremors, turning, blinking and digging (significantly different from the signs produced by DMSO–vehicle) in the SN, whereas none of these abstinence signs (or any other sign) is of significance in the DR.

Figures 2 and 3 illustrate, respectively, the effects of the focal administration of FLU and DMSO–vehicle into the DR and the SN in DZ-dependent rats on the power of slow (4–12

TABLE 1

COMPARISON OF ABSTINENCE SCORE AND SCORES FOR SOME INDIVIDUAL SIGNS OF ABSTINENCE PRECIPITATED BY FOCAL ADMINISTRATION OF FLUMAZENIL (FLU) (25 μg) INTO NUCLEUS OF DORSAL RAPHE (DR) AND SUBSTANTIA NIGRA PARS RETICULATA (SN) IN RATS CHRONICALLY EXPOSED TO DIAZEPAM (DZ) SLOWLY RELEASED FROM SILASTIC CAPSULES (540 mg/wk)

	DR	SN
Abstinence score	1.02 ± 1.84 (n.s.)	7.77 ± 1.91 ($p < 0.025$)
Tremors	0.02 ± 0.28 (n.s.)	1.65 ± 0.44 ($p < 0.025$)
Turning	0.60 ± 1.92 (n.s.)	4.02 ± 1.42 ($p < 0.05$)
Tachypnea	0.90 ± 4.85 (n.s.)	11.92 ± 4.87 ($p < 0.05$)
Blinking	0.10 ± 0.27 (n.s.)	1.62 ± 0.27 ($p < 0.005$)
Digging	−4.05 ± 6.60 (n.s.)	2.87 ± 1.08 ($p < 0.05$)

Data are normalized for between rats difference in pre-injection baseline scores and are presented as the mean area under the time course (0 – 40 min) ± SEM of 5 rats in each group. Values in parentheses indicate levels of statistical significance in comparison to DMSO-vehicle (*t*-paired test).

Hz) and fast (18–26 Hz) frequency bands. Changes in these frequency bands are commonly used to characterize BZ effect on the EEG. The EEG signals were recorded from the sites of injections (SN or DR; Figs. 2A, 3A) and from the other brain areas such as H (Figs. 2B, 3B) and FC (Figs. 2C, 3C). The data are presented as relative changes in power indicated by the decrement or increment over individual pre-FLU or pre-DMSO baseline.

The focal administration of either FLU or DMSO–vehicle into the DR produces a temporary (5 min) but pronounced enhancement of the power of 4–12 Hz and 18–26 Hz waves at the site of injection (Fig. 2). Thus, the effect of the antagonist must be considered against the effect of its vehicle. The increase of power tends to be greater for DMSO than for FLU, which suggests that FLU rapidly but briefly decreases the power of the EEG in the DR (net effect of FLU). Subsequently, the power of the slow frequency band tends to be reduced with respect to baseline in the DR, whereas it gradually decreases toward baseline in the other brain loci (H and FC). Within 10 min after injection, the effect of FLU and DMSO–vehicle on the power of the fast frequency band is not meaningful in either the DR or in the H and FC.

A different picture emerges after focal injections are made into the SN (Fig. 3). Within 5 min, both FLU and DMSO–vehicle produce an increase in power of the 4–12-Hz waves in the SN, H and FC. However, in about 15 min, the power of the slow frequency waves in these brain areas decreases and remains suppressed to the end of the EEG recording. The effect of focal injections of FLU and its vehicle on the fast frequency band is different. The relative power of the 18–26-Hz waves is reduced with respect to baseline during the whole 40-min period of post-FLU recording of the EEG. The effect of DMSO–vehicle on the fast frequency band is negligible.

The same patterns of change in power of slow and fast frequency bands are also observed in the DR and in the SN for 1–4-Hz and 12–18-Hz frequency bands, respectively (data not shown).

Figure 4 presents the time course of relative changes in total power (1–32 Hz) of the EEG in sites of focal injections (DR or SN; Fig. 4A), H (Fig. 4B) and FC (Fig. 4C) in DZ-dependent rats. The time curves for relative changes in total power are similar following injections of FLU and DMSO–

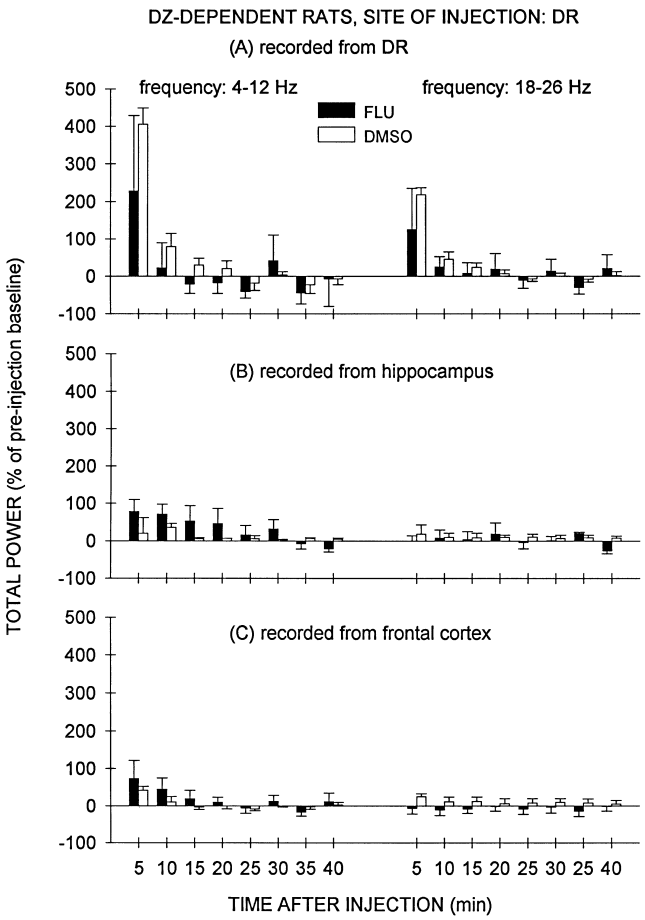

FIG. 2. The time course of changes in power (% of individual preinjection baselines) for 4–12-Hz and 18–26-Hz frequency bands following focal administrations (1 μl) of flumazenil (FLU; 25 μg) into the dorsal raphe (DR) in rats chronically exposed to diazepam (DZ) slowly released from silastic capsules (540 mg/week). The EEG was recorded from the DR (A), hippocampus (B) and frontal cortex (C). Data are the mean ± SEM of 5 rats.

vehicle into the DR, and these curves can be described by a rapid increase in total power (this effect is stronger in the DR and relatively weaker in H and FC) followed by a gradual decrease in total power toward the preinjection baseline. In contrast, the injection of FLU into the SN has a different effect on the total power of the EEG than the injection of DMSO–vehicle. The rapid but transient increment of total power of the EEG after administration of either FLU or DMSO–vehicle into the SN is followed by a FLU- but not a DMSO-induced decrement of total EEG power over preinjection baseline.

DISCUSSION

The present study examined the abstinence effect produced by the central BZ receptor antagonist FLU focally administered into the DR and into the SN in two groups of female rats chronically exposed to DZ slowly released from silastic capsules. The data show regional heterogeneity in the responses of these two brain areas to identical DZ treatment as manifested by the ability of FLU to produce a significant abstinence score in the SN pars reticulata but not in the mid-

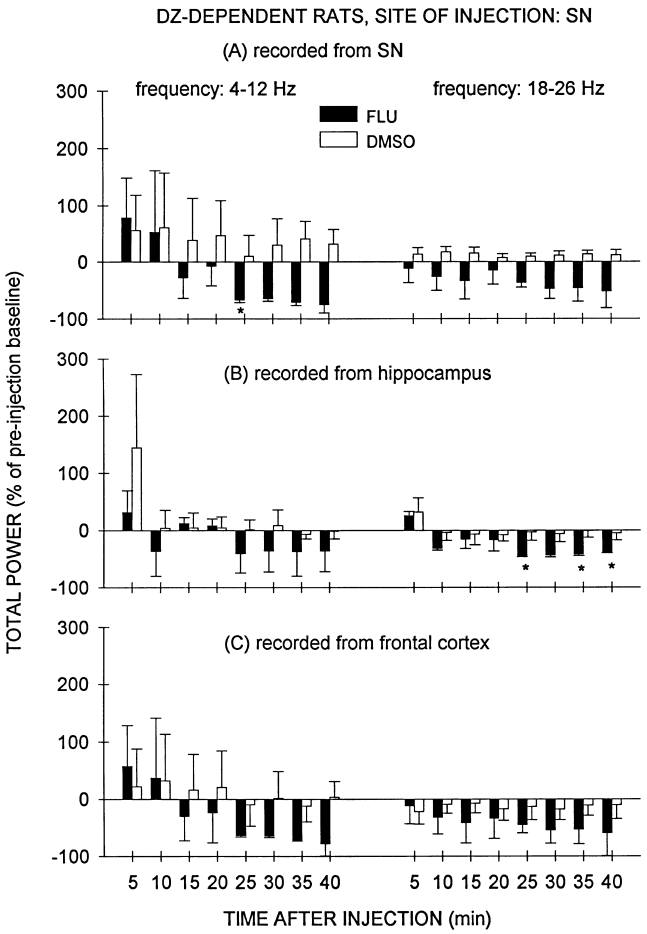

FIG. 3. The time course of changes in power (% of individual preinjection baselines) for 4–12-Hz and 18–26-Hz frequency bands following focal administrations (1μl) of flumazenil (FLU; 25 μg) and DMSO–vehicle into the substantia nigra pars reticulata (SN) in rats chronically exposed to diazepam (DZ) slowly released from silastic capsules (540 mg/week). The EEG was recorded from the SN (A), hippocampus (B) and frontal cortex (C). Data are the mean ± SEM of 5 rats. Asterisks indicate significant difference from DMSO–vehicle ($p < 0.05$, paired t-test).

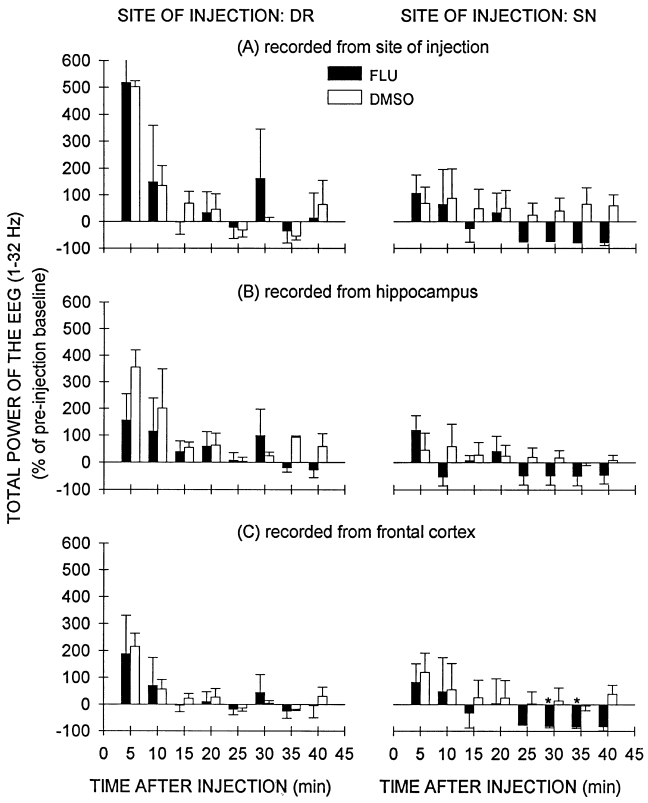

FIG. 4. The time course of changes in total power of the EEG (1–32 Hz; with respect to individual preinjection baselines) following focal injections (1 μl) of flumazenil (FLU; 25 μg) and DMSO–vehicle into the dorsal raphe nucleus (DR) and the substantia nigra pars reticulata (SN) in rats chronically exposed to diazepam (DZ) slowly released from silastic capsules (540 mg/week). The EEG was recorded in sites of injections (DR or SN; A), hippocampus (B) and frontal cortex (C). The data are presented as the mean ± SEM of 5 rats in each group. Asterisks indicate significant difference from DMSO–vehicle ($p < 0.05$; paired t-test).

brain DR nucleus. The focal injections of FLU in control rats does not have an effect. The capsule implantation technique employed in the present study (3 weeks exposure to DZ implants) produces a high level of physical dependence in rats as indicated by seizures precipitated by intravenously administered FLU [31,52,62 and unpublished data from our laboratory].

Different effects of chronic DZ treatment on the neurons of the SN and the DR have been demonstrated in a series of electrophysiological studies. In this regard, male rats that receive prolonged treatment with DZ show a decrease in postsynaptic sensitivity to iontophoretically applied GABA in serotonergic neurons within the DR and the ability of subsequently administered BZ to potentiate GABA sensitivity. A temporal relationship between the onset of GABAergic subsensitivity in the DR and the development of tolerance to the anticonvulsant effects of DZ (bicuculline seizure threshold) has been reported (21). Systemically administered FLU rapidly and persistently reverses GABA subsensitivity in the DR in rats chronically exposed to DZ but not in control rats. In both DZ-treated and control rats, the baseline firing rate of serotogenic cells within the DR is not altered by either chronic DZ treatment or acute administration of FLU (15,16,20,61). In contrast, the same chronic treatment with DZ implants does not change the sensitivity to GABA in reticulata neurons of the SN, and the continued presence of DZ potentiates the GABAergic response. Similar firing of reticulata neurons is observed in control and DZ-implanted rats. FLU, which has no effect on GABA sensitivity and on the firing rate of reticulata neurons in control rats, decreases GABA sensitivity and produces a significant overshoot in rats chronically exposed to DZ (60,63,64). It is interesting that in the SN pars reticulata in rats chronically administered flurazepam, a dose-related suppression of neuronal activity (50) and tolerance to FLU-induced circling (48) have been observed.

The data from the previous electrophysiological and the present pharmacological experiments indicate that in the DR nucleus, FLU-induced restoration of GABA sensitivity back to control levels is not accompanied by FLU-evoked abstinence signs. The GABAergic mechanism in the DR may not be directly responsible for the hyperexcitability observed dur-

ing either precipitated or spontaneous withdrawal (7,20). However, the FLU-induced increase in the firing rate of reticulata neurons is likely to be manifested by a FLU-precipitated abstinence syndrome in the SN. Thus, the level of GABA sensitivity in the DR and the SN may determine the outcome of the FLU effect at these sites in DZ-dependent rats.

It must be emphasized, however, that the present experiments were performed on female rats, whereas the data cited above were collected in male rats. Fluctuation of ovarian steroids has an impact on the behavioral and neurochemical responses of the BZ receptors (3). Although $GABA_A$ receptor parameters do not differ over the estrous cycle in rats, exogenous gonadal hormones alter the $GABA_A$/BZ/ionophore complex and GABA-mediated functions in a region-specific way [for review, see (56)]. Neither sex nor gonadectomy modified GABA sensitivity or BZ responsiveness in the SN; however, in gonad-intact male rats, the basal nigral firing rate is higher than that in gonad-intact female and in gonadectomized female and male rats (57). Unlike DZ-dependent gonad-intact male rats, the SN of ovariectomized rats does not show a reduction in responsiveness to systemically administered DZ or an increase in the firing rate with administration of FLU (55). Chronic BZ treatment results in regional differences in GABA activation of chloride flux in intact male and castrated female rats (59). During chronic exposure to DZ implants, tolerance to the anticonvulsant effects of DZ (bicuculline seizure thresholds) is observed in both gonad-intact male and female rats but not in ovariectomized rats (58). Preliminary results from our laboratory suggest that male rats develop a higher level of physical dependence on a high dose of DZ than do female rats as indicated by the abstinence syndrome precipitated by systemically administered FLU (44). Accordingly, studies on sex-related differences in the focally evoked abstinence syndrome have been initiated in our laboratory.

Surprisingly, the intranigral administration of FLU does not precipitate convulsive signs of abstinence. Preliminary data from our laboratory indicate that in rats exposed to identical chronic DZ treatment, clonic and tonic–clonic seizures and/or twitches and jerks are focally evoked by FLU in other brain areas (i.e., hippocampus, amygdala, striatum) (42,43,53). Intranigral injections of BZs produce a dose-related anti-PTZ effect that can be reversed by the concomitant focal administration of FLU (68). The SN pars reticulata has been implicated in the control of propagation of seizures (11,24,33,37), but the time course of seizures evoked by systemically administered FLU does not parallel the physiological response of reticulata neurons, which suggests that the FLU-enhanced firing rate in the SN facilitates propagation of seizures that are initiated in another brain area (62). This notion seems to be supported by the present data.

In the present study, the regional brain heterogeneity in response to chronic DZ treatment is also manifested by changes produced by FLU in the EEG. Surprisingly, DMSO–vehicle has a brief but a strong enhancing effect on the power of the EEG in the DR and a weaker effect in the SN. Although the reason for this is not known at present, this phenomenon may be due to different stress reactions in the DR and the SN, brain areas with different neural input. The vehicle (containing propylene glycol, benzoic acid and ethyl and benzyl alcohols) does not affect dopamine firing in the SN (12); the effect of this (or any other) solvent on the DR is not known. Direct application of DMSO may affect serotonin receptors and the firing of the DR neurons. Furthermore, stress-induced alteration of the GABA/BZ system may account for temporary enhancement of the power of the EEG. There is a body of literature on the impact of acute stress on GABA, serotonin and other neurotransmitters [for review, see (8)]. The pharmacological effect of DMSO should also be considered. Decreased locomotor activity has been reported after acute intraperitoneal administration of DMSO in drug-naive mice (4), whereas chronic intraperitoneal administration of DMSO results in desynchronization of the EEG in the FC and H in the rat (1). Regional heterogeneity in the effects of other solvents on the EEG has also been reported. For example, the intraperitoneal injection of saline has a weaker effect on the power of the low frequency band in FC and reticular formation than in the thalamus or striatum (6), whereas intravenously administered dimethylacetamide vehicle produces a reduction of amplitude of high frequency band in cortex (28).

In general, the present data show that in the DR, where focal application of FLU does not evoke an abstinence syndrome, FLU-produced relative changes (with respect to baseline) in the power of slow and fast frequency bands and in the total power of the EEG in either the site of injection (DR) or the other brain areas (FC and H) are not significantly different from those observed after DMSO–vehicle. In contrast, intranigral injections of FLU and its vehicle produce different effects. FLU rapidly induces an abstinence syndrome that is accompanied by a rapid decrease (with respect to the preinjection baseline) of the power of the fast frequency band and a delayed decrease of the power of the slow frequency band in the SN and in the H and FC. Taking into consideration the DMSO-induced enhancement in power of the 4–12-Hz frequency band (Fig. 3), it can be seen that changes in the total power of the EEG (Fig. 4) most likely reflect the net effect of a rapid increase in the power of the slow waves produced by DMSO–vehicle and the FLU-induced decrease in power of fast waves.

In DZ-dependent rats, FLU-induced changes in the EEG persist longer than the behavioral manifestation of the abstinence syndrome. The time course of precipitated abstinence is in concordance with the pharmacokinetics of FLU in the brain (half life = 16 min) (26), and it seems to reflect rapid displacement of DZ (metabolites) from the receptor(s). In contrast, the long time course for the EEG changes suggests that the site of injection (SN) can produce a prolonged output after the focal stimulation is over. The significance of this delay is unclear. Behavioral and EEG manifestations of the abstinence syndrome, initiated by intranigral injection of FLU, reflect a chain of functional changes that occur not only in the injection site but also in the efferent brain areas. In humans, FLU has been reported to reverse midazolam sedation rapidly; however, in the high frequency band, differences in depth of equivalent dipoles are observed immediately and up to 4 h after injection of FLU, which suggests that brain electrical activity is generated in different brain structures before and after application of BZ antagonist (5). In view of the regional heterogeneity of receptor subtypes and functional $GABA_A$/BZ coupling, different actions of BZ may be mediated by different receptor subunits present in a given brain region. In the SN, the time course of the decreased sensitivity to muscimol does not correspond with the time course for the development and reversal of tolerance to FLU (rotational behavior) or downregulation of the BZ receptors, which suggests that maintenance of GABAergic subsensitivity does not require occupation of BZ receptor(s) (48). The present data indicate that the prolonged EEG effect is observed in the SN and in cortex, where chronic DZ treatment decreases α_1 expression (23), or in the H, where subsensitivity to GABA ago-

nist and tolerance to DZ seem to follow a similar time course (67) and where chronic DZ treatment does not downregulate BZ receptors (66) but produces decreases in α_1 and α_5 mRNA levels (65). In the H, changes in the power of the fast frequency band become significant late in time, when abstinence signs are no longer observed. Acute administration of DZ results in long-lasting enhancement of fast EEG activity in the H (45). A decrease in GABA-mediated inhibition has been reported in the H in rats chronically exposed to FLU (67). Recent data from our laboratory indicate that in DZ-dependent rats focal application of FLU into CA1 area of H produces alteration of the EEG.

The exact pharmacological correlates of BZ-induced EEG changes in the slow and fast frequency bands are not completely elucidated because the relationship between alterations of the EEG and demonstration of anxiolitic, anticonvulsant or sedative properties of BZ have been reported [for review, see (27)]. However, development of tolerance to the sedative effect of chronically administered DZ is accompanied by a predominance of fast over slow wave activity in the EEG (25,32,34,51). Data from our laboratory are in agreement with the studies cited and indicate that, compared with empty-capsule controls, chronic exposure of rats to DZ implants has an influence on the spectral content of the EEG at different electrode sites (H, FC, thalamic nucleus, cerebellar cortex) as manifested by increased contribution of fast (12–32 Hz) frequency bands (accompanied by reduced contribution of low, i.e., 1–12 Hz, frequency bands) in the total power of the EEG (54). Discontinuation of DZ treatment results in a gradual decrease in the power of the fast waves toward the pretreatment baseline (22,30,39). In rats, intravenously administered FLU, which itself has no effect on the EEG, reverses the EEG changes produced by BZ agonists [for review, see (27)] and, as indicated by pharmacokinetic–pharmacodynamic modeling, the EEG changes in the 11–30-Hz frequency band suggest a BZ–FLU interaction at the GABA/BZ receptor complex (28,29). Thus, the effect of FLU on the fast frequency waves (12–32 Hz) of the EEG emphasizes its antagonistic action in the SN.

In summary, the present data indicate a different involvement of the SN and the DR in physical dependence on DZ as manifested by the precipitated abstinence syndrome and by alteration of the EEG. This result confirms the regional heterogeneity in the response to chronic BZ treatment previously demonstrated by several other measures.

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