Pergamon

Pharmacology Biochemistry and Behavior, Vol. 52, No. 4, pp. 771–778, 1995

0091-3057/95 $9.50 + .00

0091-3057(95)00177-8

# The Effect of Acute and Chronic Diazepam Treatment on Stress-Induced Changes in Cortical Dopamine in the Rat

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Received 15 March 1993

HEGARTY, A. A. AND W. H. VOGEL. *The effect of acute and chronic diazepam treatment on stress-induced changes in cortical dopamine in the rat.* PHARMACOL BIOCHEM BEHAV **52**(4) 771–778, 1995.—The mesocortical dopamine system is thought to play an important role in the etiology of the stress response. Dopamine (DA) has been shown to accumulate in the rat frontal cortex in response to a wide variety of stressors. Diazepam, an anxiolytic benzodiazepine, can reverse the effects of stress on cortical DA. We investigated the effects of acute and chronic diazepam administration on immobilization stress-induced changes of the DA system in the frontal cortex of the rat. In the first study, 2.5 mg/kg diazepam was administered 20 min prior to 40 min of immobilization stress. Acute diazepam significantly reduced basal levels of extracellular DA and antagonized the stress-induced increase in cortical DA when compared to untreated stressed rats. Acute diazepam did not significantly effect extracellular DOPAC. In the second study, an experimental group of rats was given approximately 2 mg/kg/day diazepam in their drinking water for 3 weeks. This treatment significantly reduced anxiety as assessed by a staircase test for anxiety. Chronic diazepam had no effect on basal levels of cortical DA. However, chronic diazepam treatment also attenuated stress-induced increases in extracellular DA when compared to untreated stressed control rats. Chronic diazepam did not affect stress-induced changes in DOPAC but it did antagonize the effects of stress on HVA. Thus, acute and chronic diazepam treatment can antagonize stress-induced activation of the mesocortical DA system. It is proposed that this effect is produced through an enhancement of GABAergic neurotransmission by diazepam. The role of the dopaminergic system during stress, anxiety, and schizophrenia is discussed.

Microdialysis Diazepam Stress Dopamine Frontal cortex Rats

DOPAMINERGIC systems are thought to play an important role in the etiology of the stress response in the central nervous system including anxiety and other behavioral and somatic responses. In particular, the mesocortical DA system appears to be preferentially activated by stress. Many studies using microdissection and postmortem analysis have found that stress will cause a decrease in whole tissue levels of cortical DA and an increase in tissue levels of its metabolite DOPAC in the frontal cortex (9,46,47). Furthermore, a number of microdialysis studies found that stress induced the release of DA and the accumulation of DOPAC in the frontal cortex (1,32). Also, conditioned fear, a mild, indirect stressor, only activates mesocortical DA neurons (14,25,30). Consistent with this view are observations that the systemic administration of DA agonists such as apomorphine in concentrations that only occupy autoreceptors and, thus, inhibit DA release results in anxiolysis in rats (28), and that administration of the DA $D_2$ receptor antagonist sulpiride can also produce anxiolytic actions (12).

At this time, it is generally felt that the activation of mesocortical DA neurons is related to the perception of "anxiety" and fear accompanying stress rather than pain or movement associated with stress paradigms (4). Because of this, the influence of anxiety-reducing drugs on stress-induced changes in mesocortical DA has been repeatedly tested. Benzodiazepines, a prototypical class of anxiolytic drugs, are the most widely studied anxiety-reducing drugs. They are thought to exert these actions through an enhancement of the activity of

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GABA receptors by increasing chloride ion flux through its associated channels (23). Even though benzodiazepines exert their anxiolytic actions by directly influencing GABAergic systems, they also influence dopaminergic systems. Using postmortem tissue analysis, acute diazepam treatment has repeatedly been shown to antagonize foot shock stress-induced changes in DA and DOPAC in the frontal cortex (16,21,37) and antagonize the increases in cortical DOPAC witnessed during conditioned fear (30) and psychological stress (34). Also, several studies have shown that the benzodiazepine antagonist Ro 15-1788 can prevent the elimination of stress-induced alterations in DA and DOPAC by benzodiazepines (4,15). Furthermore, administration of the anxiogenic β-carboline FG-7142, a benzodiazepine inverse agonist, induces changes in dopaminergic terminal areas that are remarkably similar to those observed during stress (4,15). However, recent studies utilizing microdialysis have shown that acute diazepam is unable to antagonize the stress-induced release of DA in the frontal cortex (20,32).

Considerably less work has been done, though, on the effects of chronic diazepam on dopaminergic systems. Chronic treatment with this drug has been shown to eliminate the fear response in a motivated discriminative task paired with shock (22) and reverse the negative effects of shock in a conditioned emotional response (39). One biochemical study was performed, which found that chronic treatment with diazepam prevented increases in tissue levels of cortical DOPAC caused by the anxiogenic β-carboline FG-7142 (29).

The following study was designed to investigate the effects of acute diazepam treatment on stress-induced changes in extracellular DA in the frontal cortex and to evaluate the effects of a 3-week exposure to this drug on the same stress-induced changes in monoamines in the frontal cortex.

## METHOD

### *Animals*

For all acute experiments, individually housed male Sprague-Dawley rats (Zivic-Miller, Allison Park, PA), weighing 225–300 g, were received 4–8 days prior to a scheduled experiment. For the chronic studies, male Sprague-Dawley rats weighing 75–150 g were received 3–4 weeks prior to the scheduled experiments. All animals were maintained in light-, temperature-, and humidity-controlled rooms on a 12 L : 12 D cycle. Food and water were available ad lib.

### *Surgery*

Bilateral dialysis probe cannulae (21 ga stainless steel, 10 mm long) were implanted during surgery under ketamine with acepromazine (1 mg/kg) and sodium pentobarbital (0.4 mg/kg) anesthesia in a Kopf stereotaxic frame. The following coordinates were used for implantation in the frontal cortex: AP +3.2, ML ±1.2, DV −1.5 (42). Animals were allowed at least 48 h to recover from surgery.

### *Microdialysis Procedure*

On the day of the experiment, a Ringer's solution composed of 145 mM NaCl, 2.7 mM KCl, 1.0 mM $MgCl_2$, 1.2 mM $CaCl_2$, and 0.2 mM ascorbate in a 2 mM phosphate buffer (pH 7.4) (38) was perfused through a concentric microdialysis probe by a syringe pump (Harvard Apparatus, South Natick, MA) fixed at a flow rate of 2.75 μl/min. Microdialysis probes (tip length 4 mm, tip diameter 0.2 mm for all experiments) were constructed from stainless steel tubing (Small Parts, Miami Lakes, FL) as described previously (8,18,26) except that the 36 ga inner cannula of the microprobe was replaced with 150 μm o.d. silica glass tubing (Polymicro Technologies, Phoenix, AZ). The glass tubing was secured with epoxy to a 30 ga stainless steel inlet tube. A Spectra/Por regenerated cellulose dialysis membrane, MWCO 6000 D (Spectrum Medical Instruments, Houston, TX) was used for all microprobes. All experiments were carried out between 0900 and 1600 h. Rats were allowed free, unrestricted movement as a result of a single fluid channel swivel system (Stoelting, Wood Dale, IL). Resting animals were perfused for 1–2 h prior to the start of the experiment, a time found to be sufficient in prior experiments (24) for a consistent, predictable output of dopamine. Experiments were started only after a stable output (± 10% variation in the DA peak between two consecutive samples) was obtained.

### *HPLC Analysis of Dialysis Samples*

Samples were collected every 20 min and analyzed immediately thereafter using high-performance liquid chromatography (HPLC) in conjunction with coulometric electrochemical detection. A DuPont (Wilmington, DE) C-18 reverse phase column (4.6 mm i.d. × 25 cm) was used with a mobile phase consisting of 0.120 M citrate, 0.110 M sodium acetate, 4.9 mM heptane sulfonic acid, 0.39 mM EDTA, and 15% methanol. A 20 μl or 55 μl aliquot (depending on the signal-to-noise ratio) was assayed for DA, DOPAC, and HVA with an ESA 5100A coulometric detector with a model 5020 guard cell and model 5011 analytical cell (Bedford, MA). The detector settings were as follows: guard cell: −0.2; detector 1: +0.35; detector 2: −0.2. The detection limit for DA in this assay was 1.0 pg/55 μl.

### *Verification of Neuronal Response With Potassium*

After a stable baseline had been reached (approximately 1–2 h), the cortex was perfused with an isoosmotic 100 mM KCl Ringer's solution for 20 min according to the technique developed by Carrozza et al. (8). Potassium-induced release can be used as an indicator of functionally active dopamine terminals in contact with the dialysis membrane. Thus, if the overflow of DA after local $K^+$ perfusion was approximately two times the basal level of DA seen prior to perfusion with the $K^+$ solution, a baseline:$K^+$ ratio of approximately 0.5, the presence of functioning dopaminergic terminals was indicated and the experiment was continued. If DA overflow remained unchanged or was less than twice the previous sample after $K^+$ perfusion, the experiment was terminated. The DA overflows resulting from repeated $K^+$ challenges were comparable whether administered before or at the end of the experiment. An experiment was continued following a $K^+$ response only after a stable baseline was reached approximating that which was obtained prior to $K^+$ perfusion.

### *Experimental Procedures*

*Effect of acute diazepam and stress in the frontal cortex.* Rats were divided into four groups for this experiment. To begin each experiment, a microprobe was inserted into the right or left cannula of an unanesthetized, unrestrained rat. Following insertion of the probe, samples were collected every 20 min. Approximately 60–80 min elapsed before DA and DOPAC reached stable baseline peaks. After two reproducible peaks were observed, a 100 mM KCl Ringer's solution was perfused through the microprobe for 20 min. If a significant increase in extracellular DA (at least twice the baseline level) and concomitant decrease in extracellular DOPAC was ob-

served in the sample collected 20 min after cessation of $K^+$ perfusion, the experiment was continued. An experiment was continued not less than 1 h after the last $K^+$ perfusion to reestablish a stable DA baseline. This procedure was repeated at the conclusion of the experiment. The first group of rats ($n$ = 7) received an injection of saline equivalent in volume to 2.5 mg/kg diazepam. The diazepam control group ($n$ = 9) received 2.5 mg/kg of a prepared, injectable diazepam solution (Elkins-Sinn, Cherry Hill, NJ). The third group of rats was immobilized for 40 min ($n$ = 13). The rats were immobilized in a prone position by taping each leg to a tabletop and wrapping a strip of terry cloth around its abdomen and securing it to the table. This procedure significantly impairs all movement with the exception of the head and tail. And the fourth experimental group ($n$ = 8) was given 2.5 mg/kg diazepam 20 min prior to 40 min of immobilization.

*Effect of chronic diazepam and stress in the frontal cortex.* Upon arrival, rats were randomly divided into two groups: a control group that received a normal diet, and a diazepam-treated group that was given a solution containing diazepam instead of untreated drinking water. The diazepam-treated rats were given 48 h to become acclimated to their new environment before the drug treatment was commenced. Thereafter, the diazepam-treated rats were given drinking water which contained 0.2 mg/30 ml of solution, or, 2 mg/kg diazepam daily. Because rats show a constant fluid intake, the amount of drug consumed can be predicted and checked the next day. Nevertheless, we checked the water intake of the first two animals that received diazepam-treated water before the start of the experiment and after the start of the experiment to ascertain any changes in drinking patterns. No alterations were observed; therefore, the amount of water consumed by each diazepam-treated rat was no longer measured to prevent investigator bias. After 3 weeks of treatment, rats were divided into four groups: resting control rats ($n$ = 7), resting diazepam-treated rats ($n$ = 6), control rats that were immobilized for 40 min ($n$ = 8), and diazepam-treated rats ($n$ = 7) that were immobilized for 40 min. To begin each experiment, a microprobe was inserted into the right or left cannula of an unanesthetized, unrestrained rat. Following insertion of the probe, samples were collected every 20 min. Approximately 60–80 min elapsed before DA, DOPAC, and HVA reached stable baseline peaks. After two reproducible peaks were observed, a 100 mM KCl Ringer's solution was perfused through the microprobe for 20 min. If a significant increase in extracellular DA (at least twice the baseline level) and concomitant decreases in extracellular DOPAC and HVA were observed in the sample collected 20 min after cessation of $K^+$ perfusion, the experiment was continued. An experiment was continued not less than 1 h after the last $K^+$ perfusion to reestablish a stable DA baseline. This procedure was repeated at the conclusion of the microdialysis experiment. Two days after completion of the microdialysis experiments, 10 control rats and 11 diazepam-treated rats were placed in a staircase box for 5 min to assess the efficacy of the chronic drug treatment. It contained three steps that were 4″ high × 5″ deep. The rat was placed at the bottom of the box, below the first step and facing away from the staircase. After the rat was released, the time it required to ascend each of the three steps and the number of ascents and descents on each step within 5 min was recorded.

### *Probe Placement Verification and Histological Analysis*

Rats were anesthetized with 0.4 mg/kg sodium pentobarbital and euthanized by transcardial perfusion with 10% formalin solution or Bouin's fixative. The brains fixed with formalin were inspected visually to confirm the correct placement of the probe in the frontal cortex. Probe placement in the cortex was confirmed if the tract lay anterior and medial to the rostral end of the corpus callosum. The brains perfused with Bouin's fixative were prepared for histological analysis to verify probe placement.

### *Statistical Analysis*

Experimental data were analyzed with a SAS/STAT (SAS Institute, Cary, NC) General Linear Models procedure consisting of a two-way repeated measures analysis of variance of time (seven levels) vs. treatment. Each analysis of variance compared two levels of treatment per test. However, the analysis was repeated so that all four treatments were compared with one another. A Bonferroni procedure was used to correct for multiple comparisons, resulting in a significant effect at $p \leq 0.017$. The analysis of variance was then followed by a Student–Newman–Keuls post hoc test for a comparison of means. The amount of DA, DOPAC, and HVA observed after $K^+$ perfusion was used as a correction factor to reduce variability in the data between subjects. This was accomplished by dividing the data for each experiment by the average $K^+$-evoked peak value determined for that animal. Baseline values were normalized to 100% and changes were calculated from this point as described previously (24). The data are presented as percentiles but log transformations of these values were performed prior to statistical analysis to eliminate the distorting effect of percentile values. Truepistat software (Epistat Sigma Service, Richardson, TX) was used for $t$-tests performed on the behavioral data.

## RESULTS

Baseline levels of DA and DOPAC in the acute experiments were 2.28 ± 0.46 and 44.03 ± 7.6 pg/20 μl, respectively. Baseline levels of DA, DOPAC, and HVA in the chronic control animals were 0.81 ± 0.21, 27.66 ± 4.4, and 140.2 ± 18.6 pg/20 μl, respectively. Baseline levels of DA, DOPAC, and HVA in the chronic diazepam-treated rats were 0.61 ± 0.17, 22.78 ± 3.7, and 138.6 ± 23.3 pg/20 μl, respectively. All concentrations are mean ± SEM.

### *The Effect of Acute Diazepam and Stress on Cortical DA*

An injection of saline had no effect on DA levels, but an acute injection of 2.5 mg/kg diazepam in resting animals significantly reduced extracellular DA 40 min after administration of the drug, $F(1, 14) = 10.95$, $p = 0.005$ (Fig. 1). This effect disappeared within 20 min. Stress caused significant increases in DA compared to both saline- and diazepam-treated animals. A significant time × treatment interaction was observed between diazepam and stress, $F(5, 100) = 3.39$, $p = 0.007$. When the same dose of diazepam was administered prior to immobilization, the stress-induced increase in DA within the first 20 min of immobilization was antagonized. However, the combined diazepam-stress treatment also prevented the sudden drop in extracellular DA observed when diazepam was given to resting rats. Overall, diazepam pretreatment eliminated the effects of stress on extracellular DA as shown by a significant time × treatment interaction, $F(5, 95) = 2.96$, $p = 0.016$. This effect, though, was much more variable during the second 20 min of immobilization.

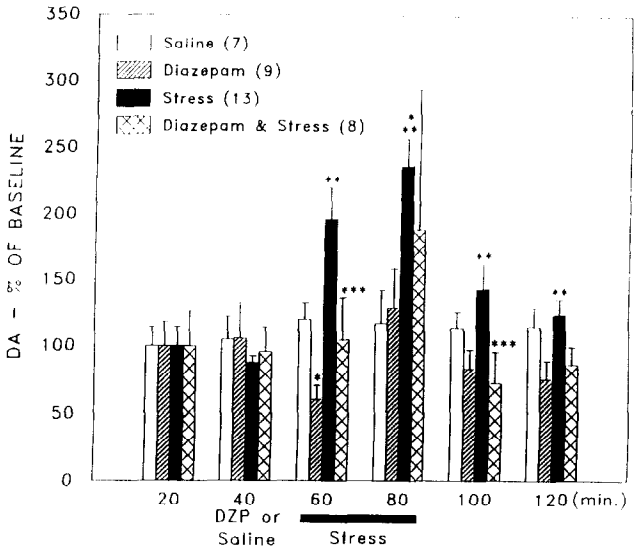


FIG. 1. The effect of 2.5 mg/kg IP diazepam on cortical DA in resting and stressed rats. Stressed rats were immobilized for 40 consecutive min. Horizontal axis labels designate samples collected 20 min after the indicated treatment. Refer to the graph legend to determine which treatments were administered to each group and the number of animals in each group. Results are expressed as the mean + SEM percent variation of the baseline: $K^+$ peak ratio. Interactions: diazepam vs. stress ($p$ = 0.007); stress vs. diazepam and stress ($p$ = 0.016). *Significant vs. saline ($p < 0.05$); **significant vs. diazepam ($p < 0.05$); ***significant vs. stress ($p < 0.05$).

*The Effect of Acute Diazepam and Stress on Cortical DOPAC*

The acute diazepam treatment did not significantly alter extracellular DOPAC in resting rats, $F(5, 75) = 0.05$, $p$ = 0.99 (Fig. 2), but it appears that diazepam may have increased DOPAC levels in some rats within 20 min of administration of the drug. Not unexpectedly, stress significantly increased DOPAC relative to the saline-treated controls, $F(5, 135)$ = 3.05, $p$ = 0.01. However, administration of diazepam antagonized and eliminated the stress-induced increase in extracellular DOPAC. A significant interaction was observed between the stress animals and the diazepam-stress treatment group, $F(5, 90) = 4.05$, $p$ = 0.002.

*The Effect of Chronic Diazepam Treatment on a Staircase Test for Anxiety*

Rats given diazepam in their water for 3 weeks were placed in a three-step staircase box for 5 min to test the anxiolytic action of diazepam. Table 1 summarizes the salient features of this test. Rats that had received diazepam for 3 weeks ascended to the first step twice as fast as the control animals and climbed to the second step three times as quickly as the control rats. These times were not significantly different from each other, though, which was probably due to the high variability among the animals. However, the number of times the diazepam-treated animals climbed up and down the first two steps was significantly greater than the corresponding exploratory behavior in the control animals. Thus, chronically treated animals are less anxious indicative of an anxiolytic effect of diazepam.

*The Effect of Chronic Diazepam Treatment and Stress on Cortical DA*

Figure 3 shows that there is not a significant effect of chronic diazepam treatment relative to the control treatment in resting rats, $F(1, 10) = 5.62$, $p$ = 0.04. When the control rats were stressed, DA increased approximately 148% over the estimated baseline value. This result is not unlike the stress response observed in the acute experiments. When the rats chronically treated with diazepam were immobilized, the characteristic increase in extracellular DA was not observed. This treatment combination was not significantly different from the diazepam-treated resting rats, $F(5, 55) = 1.54$, $p$ = 0.19, or the control resting rats, $F(5, 55) = 0.38$, $p$ = 0.86, at any time. The changes in extracellular DA observed in the stressed, diazepam-treated rats was of a smaller magnitude than that which was observed in the stressed control rats and there was a significant time × treatment interaction between the two groups of stressed animals, $F(5, 60) = 3.06$, $p$ = 0.016. Thus, chronic diazepam treatment did not cause marked changes in extracellular DA in resting rats but significantly reduced the effects of stress on extracellular DA.

*The Effect of Chronic Diazepam Treatment and Stress on Cortical DOPAC*

Chronic diazepam treatment did not significantly change the level of extracellular DOPAC in resting rats, $F(5, 80)$ = 1.78, $p$ = 0.13 (Fig. 4). Immobilization of the control animals increased DOPAC over the levels of both control and diazepam-treated resting rats, but this effect only reached statistical significance for the control group, $F(5, 85) = 4.49$, $p$ = 0.001, and $F(5, 105) = 2.31$, $p$ = 0.05, respectively. How-

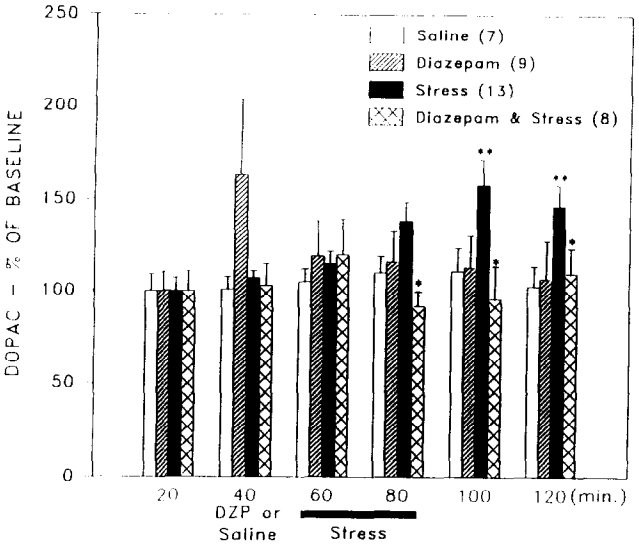


FIG. 2. The effect of 2.5 mg/kg IP diazepam on cortical DOPAC in resting and stressed rats. Stressed rats were immobilized for 40 consecutive min. Horizontal axis labels designate samples collected 20 min after the indicated treatment. Refer to the graph legend to determine which treatments were administered to each group and the number of animals in each group. Results are expressed as the mean + SEM percent variation of the baseline: $K^+$ peak ratio. Interactions: stress vs. saline ($p$ = 0.01; stress vs. diazepam and stress ($p$ = 0.002). *Significant vs. stress ($p < 0.05$); **significant vs. saline ($p < 0.05$).

TABLE 1

SUMMARY OF RESULTS OF A STAIRCASE TEST FOR THE ANTIANXIETY EFFECTS OF CHRONIC DIAZEPAM TREATMENT

| | Control | Diazepam | $p$ |
|---|---|---|---|
| Delay – step 1 | 40.1 s | 19.5 s | NS |
| Delay – step 2 | 93.2 s | 30.7 s | NS |
| Delay – step 3 | 162 s | 153 s | NS |
| No. of times – step 1 | 6.8 | 11.4 | 0.03 |
| No. of times – step 2 | 3.6 | 6.5 | 0.05 |
| No. of times – step 3 | 1.1 | 2.1 | NS |

Delay is the amount of time in seconds spent in the staircase box before before climbing completely onto the next higher step. No. of times is a count of the ascents and descents onto each step within five minutes. Control: includes stressed and unstressed animals ($n$ = 10); diazepam: includes stressed and unstressed animals ($n$ = 11).

ever, chronic diazepam treatment did not reverse stress-induced increases in extracellular DOPAC. In fact, a significant effect of time for both control and diazepam-treated stressed rats was observed, $F(5, 100) = 7.95, p = 0.0001$.

*The Effect of Chronic Diazepam Treatment and Stress on Cortical HVA*

Similar to DOPAC, chronic diazepam treatment had no measurable effect on extracellular HVA in resting animals, $F(5, 55) = 0.37$, $p = 0.87$ (Fig. 5). The stress-induced increase in extracellular HVA seen in the control animals did not have a significant effect with respect to resting, control animals, $F(5, 60) = 0.68, p = 0.64$, or diazepam-treated resting rats, $F(5, 65) = 1.52$, $p = 0.19$. The diazepam-treated rats did not respond to stress with the same increase in HVA, but there was a significant effect of time in both groups of stressed rats, $F(5, 60) = 4.38, p = 0.002$.

## DISCUSSION

Under these experimental conditions, a single injection of diazepam into resting rats significantly reduced extracellular DA 40 min after injection. Other studies have reported this same phenomenon (20,32), and decreased DA utilization has been observed in tissue homogenates (5,11). However, the decrease in extracellular DA was reversed when animals were immobilized 20 min after receiving diazepam. Diazepam now significantly attenuated stress-induced increases in DA in the first 20 min, but this effect was no longer apparent during the second 20 min of immobilization. This finding contradicts other microdialysis studies using the same dose of diazepam (20,32); however, these investigators waited 1 h after administration of diazepam before stressing the rat, whereas 20 min elapsed between drug treatment and immobilization in our experiment. Diazepam was able to significantly antagonize the stress response during the first 20 min of immobilization but not during later times. Also, this study used an injectable diazepam solution that might have effects other than those observed with pure diazepam, though this seems unlikely (49).

There are several studies that support our conclusion that acute diazepam can, indeed, reverse the effects of acute stress. Biggio et al. (4) have concluded that the tonic GABAergic modulation of the cortex is adversely affected by acute stress in a naive rat. Here, acute stress decreased both basal $^{36}Cl^-$ flux and the density of low affinity $GABA_A$ receptors in the brain. This was confirmed by demonstrating that the $GABA_A$ receptor antagonist bicuculline causes the same results as exposure to foot shock. Thus, it is possible that an enhancement of GABAergic neurotransmission by diazepam can reverse these negative effects on the function of $GABA_A$ receptors. However, it is also possible that diazepam treatment can only enhance GABAergic transmission to such an extent that the function of GABA neurons can only return to basal levels and not increase GABAergic transmission to a point where the effects of stress are completely reversed. This might explain the results seen in several studies in which the effects of stress were not reversed by pretreatment with diazepam (20,32).

The effects of diazepam on extracellular DA in resting and stressed rats might be due to its effects on A10 neurons in the ventral tegmental area (VTA) that innervate the frontal cortex. It has been suggested that acute diazepam treatment might enhance GABAergic neurotransmission in the VTA (37) or decrease GABAergic influence on VTA DA neurons (41). Perhaps both of these conclusions are true. O'Brien and White (41) found that intravenously administered diazepam inhibited non-DA VTA neurons and disinhibited DA VTA neurons, whereas microiontophoretically administered diazepam inhibited non-DA VTA neurons but had no affect on DA VTA neurons. They concluded that the effects of diazepam on DA VTA cells must be an indirect excitation of these cells as a result of the inhibition of inhibitory non-DA interneurons within the VTA. This conclusion is supported by their results for iontophoretic application of diazepam, which indicate that benzodiazepine receptors are only found on non-DA VTA cells (41). However, the results presented here along with previous studies (5,11,32) attest to a decrease in the activity of dopaminergic neurons innervating the cortex, a phenomenon

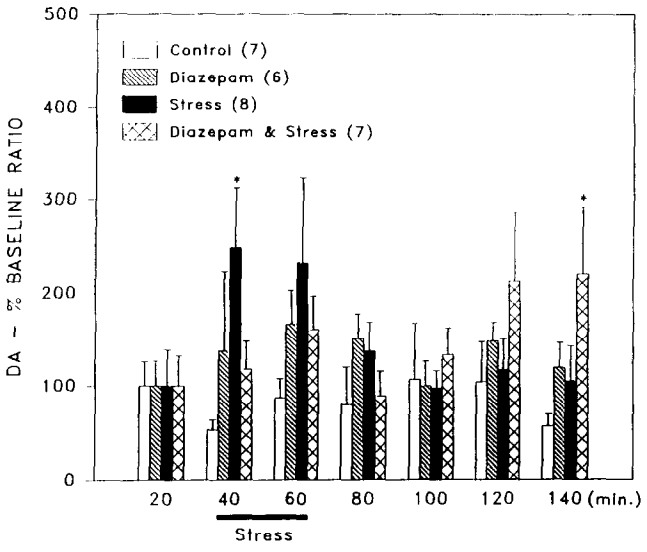


FIG. 3. The effect of chronic diazepam treatment on cortical DA in resting and stressed rats. Stressed rats were immobilized for 40 consecutive min. Horizontal axis labels designate samples collected 20 min after the indicated treatment. Refer to the graph legend to determine which treatments were administered to each group and the number of animals in each group. Results are expressed as the mean + SEM percent variation of the baseline: $K^+$ peak ratio. Interactions: stress vs. diazepam and stress ($p$ = 0.016). *Significant vs. control ($p < 0.05$).

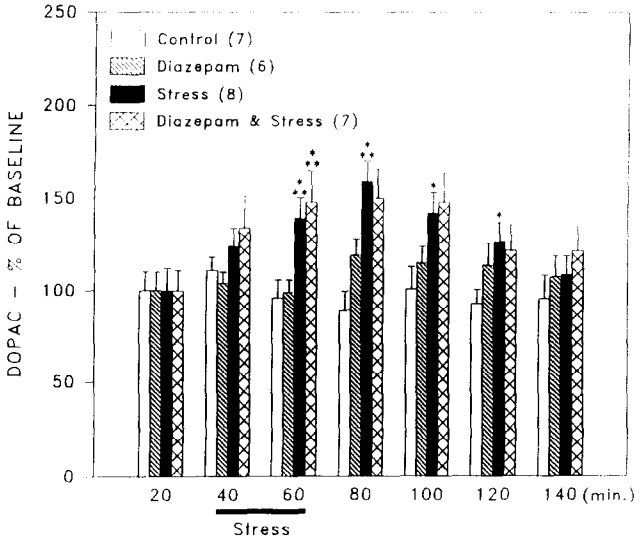


FIG. 4. The effect of chronic diazepam treatment on cortical DOPAC in resting and stressed rats. Stressed rats were immobilized for 40 consecutive min. Horizontal axis labels designate samples collected 20 min after the indicated treatment. Refer to the graph legend to determine which treatments were administered to each group and the number of animals in each group. Results are expressed as the mean + SEM percent variation of the baseline: $K^+$ peak ratio. Interactions: control vs. stress ($p = 0.001$). *Significant vs. control, diazepam ($p < 0.05$); **significant vs. control ($p < 0.05$).

that is supported by other electrophysiological studies that have demonstrated a benzodiazepine-induced decrease in multiunit activity in the substantia nigra and other central nuclei (36,44). Immobilization stress reduced the ability of diazepam to decrease the activity of dopaminergic neurons in the frontal cortex. This effect might be due to the sharp decline in benzodiazepine binding (38) and/or GABAergic function (4) that can occur during acute stress. This would account for the reversal of the antistress effect of diazepam observed 20 min after the onset of stress and, perhaps, explain the consistent reports of the efficacy of much higher doses of diazepam (5–10 mg/kg) in reversing the effects of stress in the cortex (17,21,30,31,34,37).

Acute diazepam had no effect on extracellular DOPAC in resting rats. Immobilization caused a small but significant increase in DOPAC, which was apparently antagonized by acute diazepam. The reduction in DOPAC may be due to the inactivation of DA neurons by systemic diazepam treatment described formerly. Interestingly, though, a sharp but nonsignificant increase in extracellular DOPAC was recorded within the first 20 min following diazepam administration. This might have been the result of an early activation of dopaminergic VTA neurons, which could increase DA synthesis and, in turn, increase production of DOPAC, thus supporting the theory proposed by O'Brien and White (41).

Three weeks of treatment with diazepam reduced anxiety as shown in the staircase test paradigm. The animals that were chronically treated with diazepam appeared to react to immobilization stress with approximately the same degree of struggling and distress as the control animals (personal observations). This is in contrast to the acute diazepam treatment that resulted in a significant reduction in the extent of struggling during immobilization relative to controls (personal observations). This is most likely a result of the differing behavioral effects of diazepam when administered either acutely or chronically. That is, acute diazepam primarily produces sedation, whereas chronic diazepam treatment leads to a tolerance to the sedative effect and a primary anxiolytic effect. Even though their behavior appeared unchanged during stress, the customary increase in extracellular DA did not accompany the stress response of the diazepam-treated rats. This supports earlier findings of a number of behavioral studies that found that chronic diazepam treatment increased responding in conditioned fear paradigms (22,39), stressors that have been shown to preferentially activate the mesocortical dopaminergic system. The present result not only indicates that the changes in cortical DA during stress may not be related to the physical response to immobilization, it also demonstrates the importance of the mesocortical dopaminergic system in the emotional states of stress-induced anxiety. For instance, if activation of the frontal cortex is associated with an attempt to cope with a stressor, as has been previously suggested (9,13,45), it is possible that diazepam has eliminated the necessity of a coping response and, as a result, the mesocortical dopaminergic system was not activated to the same extent to which it was activated during stress.

The effect of chronic diazepam on dopaminergic neurons might also be mediated through GABAergic modulation of DA VTA neurons. Diazepam and other benzodiazepines increase GABAergic neurotransmission by increasing $^{36}Cl^-$ conductance through benzodiazepine receptors (23). Also, chronic treatment with benzodiazepines can enhance the effects of GABA (27,51) and reverse the effects of the anxiogenic compound FG-7142 on the mesocortical DA system (29). However, the relationship that GABAergic and mesocortical dopaminergic systems have to stress and anxiolysis is still unclear. It has been proposed that descending GABAergic path-

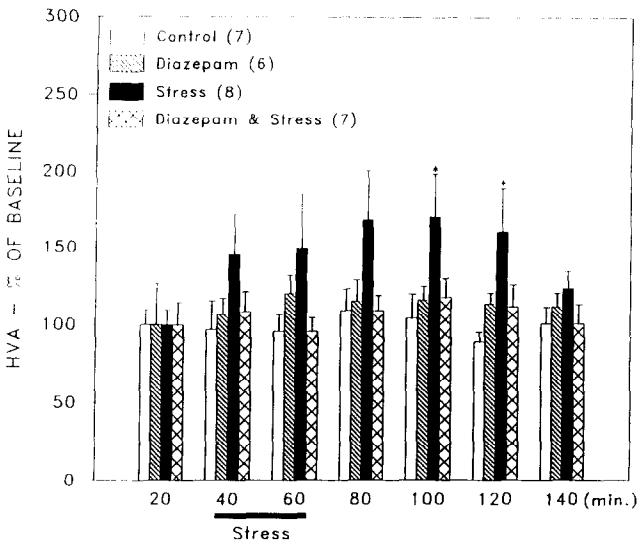


FIG. 5. The effect of chronic diazepam treatment on cortical HVA in resting and stressed rats. Stressed rats were immobilized for 40 consecutive min. Horizontal axis labels designate samples collected 20 min after the indicated treatment. Refer to the graph legend to determine which treatments were administered to each group and the number of animals in each group. Results are expressed as the mean + SEM percent variation of the baseline: $K^+$ peak ratio. *Significant vs. control ($p < 0.05$).

ways exert tonic inhibitory control over non-DA inhibitory interneurons in the VTA (41). If this is true, the chronic disinhibition of DA VTA neurons caused by increased GABAergic neurotransmission during diazepam treatment may alter their responsiveness to an acute stressor. These results support the hypothesis that diazepam has a significant, though indirect, influence on the mesocortical dopaminergic system as a result of the tonic control GABAergic neurons have on cortically projecting DA VTA neurons.

The results of our chronic study might also have important implications in the treatment of schizophrenia. Stress has been claimed to be associated with the onset and exacerbation of schizophrenia (7). Because of this observation, benzodiazepines have been used as adjuncts to neuroleptic treatment. However, the actual efficacy of this combined treatment has remained in doubt as a result of ambiguous findings of several clinical studies (7). However, in a more recent study, Breier et al. found that alprazolam given in conjunction with fluphenazine significantly reduced symptom ratings relative to those observed with fluphenazine treatment alone, indicating that benzodiazepine treatment does have some influence on psychotic symptoms (7). The significance of the present study to the treatment of schizophrenia includes the relationship between stress and the activity of dopaminergic systems. Because the excessive activity of dopaminergic systems is thought to be involved in the etiology of schizophrenia (43), the activation of dopaminergic systems by stress may lead to observed psychotic relapses. And, even though neuroleptics block the activity of dopaminergic neurons, they may not prevent stress-induced activation of dopaminergic systems. In support of this theory, metabolic stress has been shown to increase plasma HVA in neuroleptic-treated schizophrenics (7). Therefore, the present study supports the use of benzodiazepines as an adjunct in the treatment of schizophrenia because diazepam prevented the stress-induced activation of the mesocortical dopaminergic system, reducing the possibility of a psychotic relapse due to stress.

Little work has been done on the effects of chronic benzodiazepine treatment on neurotransmitter systems other than the GABAergic system. This makes it difficult to interpret the results of chronic diazepam treatment on monoamine metabolites. Finlay et al. found that DOPAC and HVA responded similarly to a midazolam challenge after chronic treatment with midazolam (19). We found differential effects on DOPAC and HVA during stress after chronic diazepam treatment. Chronic diazepam treatment did not alter the effects of stress on cortical DOPAC but it did antagonize the effects of stress on HVA, a finding that has also been observed in acute studies (6) and humans (7). DOPAC is produced within the nerve terminal and HVA is primarily produced in the synaptic cleft (10). Thus, even though the effects of stress on DA release are reversed, stress may still be affecting a mechanism that leads to the accumulation of DOPAC but has little or no influence on extracellular HVA.

In this study, we used acute implantation of dialysis probes in conscious animals instead of chronic implantation, i.e. > 24 h. Although we tested for the presence of functioning dopaminergic nerve terminals with infusions of KCl before and after the experiment, it is possible that neurotransmitter efflux measured less than 24 h after implantation of a dialysis probe may not be of neuronal origin (3). However, a number of previous studies have demonstrated that dopaminergic terminals respond to a variety of drug treatments following acute implantation of the probe. For example, apomorphine has been shown to completely block the release of DA following acute implantation (52), and an extensive study in the striatum demonstrated that DA release from nigrostriatal terminals was almost completely blocked after perfusion with tetrodotoxin or EGTA (2). In the same experiment, robust increases in extracellular DA were observed after perfusion with $K^+$ and amphetamine, among others (2). These findings indicate that at least some of the alterations in DA we measured in this study were probably due to release of DA from functioning terminals.

We have found that both acute and chronic diazepam treatment can antagonize stress-induced alterations in the mesocortical dopaminergic system. These findings support an important role for the dopaminergic system in stress and anxiety and perhaps schizophrenia as well.

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