Pharmacology Biochemistry and Behavior, Vol. 66, No. 3, pp. 585–590, 2000

0091-3057/00/$–see front matter

ELSEVIER

PII S0091-3057(00)00255-0

Dexamethasone Reverses the Ethanol-Induced Anxiolytic Effect in Rats

V. M. M. FERREIRA, R. N. TAKAHASHI AND G. S. MORATO

Departamento de Farmacologia, Universidade Federal de Santa Catarina, Florianópolis, SC, 88015-420, Brazil

Received 23 August 1999; Revised 23 November 1999; Accepted 31 December 1999

FERREIRA, V. M. M., R. N. TAKAHASHI AND G. S. MORATO. *Dexamethasone reverses the ethanol-induced anxiolytic effect in rats.* PHARMACOL BIOCHEM BEHAV **66**(3) 585–590, 2000.—The effects of intraperitoneal and intrahippocampal administration of the glucocorticoid dexamethasone were assessed regarding ethanol-induced anxiolysis in the elevated plus-maze in rats. Animals pretreated with systemic injections of dexamethasone (0.5, 1.0, or 2.0 mg/kg, IP) 15 min before ethanol (1.2 g/kg, 14% w/v, IP) administration showed a significant dose-dependent attenuation of the increased percentage of frequency and time spent on open arms of the maze. However, IP dexamethasone treatment 4 h before the test had no effect. Unilateral intrahippocampal injection of dexamethasone (2 and 20 nmol in 0.5 μl) also significantly attenuated the increased exploration of the open arms induced by ethanol. The results are interpreted in terms of the modulation of the anxiolytic effects of ethanol by glucocorticoids and the possible involvement of hippocampus in this response. The rapid blockade of ethanol induced anxiolysis by dexamethasone strengthens the suggestion that a nongenomic mechanism may underlie this response. © 2000 Elsevier Science Inc.

Ethanol Anxiety Dexamethasone Hippocampus

ETHANOL affects the central nervous system (CNS) function by several different mechanisms, such as the induction of primary perturbation of neuronal membranes (13), reduction of calcium influx through voltage-sensitive calcium channels (38), and alteration in the release of the neurotransmitters dopamine (16), acetylcholine (7), norepinephrine (28), and serotonin (35). In recent years, emphasis has been given to the studies involving the actions of ethanol on the amino acid neurotransmitter systems. There is evidence that ethanol can either stimulate the $GABA_A$ receptor or inhibit NMDA receptor activation (17,22).

A variety of evidence has associated increased ethanol consumption and anxiety-related behavior. Clinical studies have suggested that ethanol reduces the states of anxiety, frustration, or stress (15,20,23,39). Ethanol also displays an anxiolytic-like profile of action in different models of anxiety (2,5,6,11). Recent studies in our laboratory have shown a clear anxiolytic effect in rats of both sexes tested on the elevated plus-maze (9,10). Moreover, rats previously classified as anxious in the elevated plus-maze exhibited higher place preference for ethanol than nonanxious rats (3).

Although basic research has been equivocal on the relationship of corticosteroids to behavioral responses in animal models of anxiety, recent work has shown that corticosteroid administration produces anxiogenic effects (29), while the administration of mineralocorticoid (MR) or glucocorticoid receptor (GR) antagonists causes anxiolytic-like effects in different anxiety tests, including the elevated plus-maze (1,18, 19). Interestingly, the literature on the interaction between the behavioral effects of ethanol and corticosteroids is limited. For example, glucocorticoids have been shown to affect the development of tolerance to and dependence on ethanol (32,33). Moreover, corticosterone increases the severity of symptoms exhibited by mice after withdrawal from ethanol and other depressant drugs (26), and adrenalectomy reduces the ethanol consumption of rats (8). In addition, a recent study has implicated ethanol in the activation of the hypothalamic–pituitary–adrenal (HPA) axis in a mouse model of binge drinking (24). Further, the hypnotic effect of ethanol in mice has been shown to be consistently antagonized by previous injection of a large dose (20 mg/kg) of the glucocorticoid dexamethasone (34). Concerning other autonomic measure-

Requests for reprints should be addressed to Dr. Gina Struffaldi Morato. Departamento de Farmacologia, Universidade Federal de Santa Catarina, Florianópolis, SC, 88015-420, Brazil.

ments, it has been shown that dexamethasone suppresses the hypertension induced by ethanol in humans (25), while our recent article has shown that ethanol-induced hypothermia in rats is antagonized by dexamethasone (4). In light of these considerations, the present study was undertaken to examine whether ethanol-induced anxiolytic effect could be influenced by pretreatment with the glucocorticoid dexamethasone, and also to ascertain whether hippocampal receptors might be involved in mediating this response.

METHOD

Animals

Male Wistar rats from our own colony, weighing approximately 310 g, 4 month old, were used. The animals were housed in groups of eight per cage, with free access to food and water, in a room maintained at 23 ± 1°C, under a 12 L:12 D cycle (lights on at 0600 h).

Drugs

Ethanol, chloral hydrate, and propilenoglicol from Merck Laboratory (Hawthorne, NY) and sodium pentobarbital (Abbott Laboratories, São Paulo, Brazil) were used to prepare the anesthetic Equitesin®. Sodium chloride (NaCl) and magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$) were supplied by the Sigma Chemical Co. (St. Louis, MO). Dexamethasone, obtained from Research Biochemical International (Natick, MA), was dissolved in 0.15 M NaCl (saline). The doses used were selected from the literature and from preliminary experiments (4,9). For the behavioral tests, ethanol was prepared by dilution in saline to the concentration of 14% w/v.

Surgery

Rats were anesthetized with Equitesin®, prepared by vigorously mixing two solutions. Solution A contained chloral hydrate (8.5 g), $MgSO_4 \cdot 7H_2O$ (4.252 g), H_2O (91.40 ml). Solution B contained sodium pentobarbital (1.94 g), EtOH (13.0 ml), and propilenoglicol (85.6 ml). The volume injected was 0.3 ml/100 g of body weight, IP. The rats were then stereotaxically implanted with a cannulae directed to the hippocampus (AP −5 mm, ML −5 mm, DV −6 mm). The cannula was anchored to the bone with stainless steel screws and acrylic cement. A stiletto was introduced inside the outer guide cannulae to prevent its obstruction. Five to 7 days after recovery from surgery, the rats were randomly assigned to the elevated plus-maze test.

Elevated Plus-Maze (EPM) and Wooden Arena

The apparatus was a wooden, plus-shaped maze, elevated 50 cm from the floor. Two opposite arms were open (50 × 10 cm), and the other two opposite arms were enclosed with walls (50 × 10 × 40 cm). To minimize falls of the animals from the open arms, these were surrounded by a 1 cm-high Plexiglas edge (21). The equipment was placed in a dark room (44 lx). The wooden arena in which rats were observed before exposure to the maze measured 60 × 60 × 35 cm. Anxiolytic effects are defined as an increase in the proportion of open arm entries divided by the total number of arm entries, and the time spent on open arms relative to the total time spent on both arms. Any decrease in these parameters indicates an axiogenic effect.

GENERAL PROCEDURE

Experiment 1—Effects of Systemic Dexamethasone Pretreatment 15 min or 4 h Prior to the Testing of Ethanol-Treated Rats in the Elevated Plus-Maze

Four groups of 20 rats each were injected with dexamethasone (0.5, 1.0, or 2.0 mg/kg, IP) or saline, respectively. Fifteen minutes after pretreatment, each group was subdivided into two groups to receive ethanol (1.2 g/kg, 14% w/v, IP) or saline (0.9% NaCl, IP). After 10 min, rats were individually placed inside the arena for 5 min and then they were placed in the center of the elevated plus-maze where their behavior was recorded over a period of 5 min. Whenever a rat placed four paws onto an arm, one entry was recorded. The frequencies of entries on the open and on the enclosed arms, as well as the time spent on each of the arms, were recorded, and the percentages of open-arm entries and of the time spent on the open arms were taken into consideration. Other groups of rats were tested under the same conditions, except that dexamethasone was injected 4 h before ethanol.

Experiment 2—Effects of Intrahippocampal Dexamethasone on the Behavior of Ethanol-Treated Rats Tested in the Elevated Plus-Maze

Two groups of rats were intraperitoneally (IP) injected with saline or ethanol (1.2 g/kg). After 10 min, each group was subdivided into three subgroups ($n = 10$) to receive saline (control group) or dexamethasone (2.0 or 20 nmol) by intrahippocampal route. A volume of 0.5 μl was injected. Ten minutes later, each rat was placed in the arena for 5 min and then was observed in the elevated plus-maze for 5 min, as described in the previous experiment.

Histology

At the completion of the testing, rats were deeply anesthetized with Equitesin®, and each rat was transcardially perfused with 0.9% NaCl followed by a 10% formaldehyde solution. A 0.5-μl microinjection of Evans Blue was applied through the guide cannulae to mark the exact location of the previous microinjection. Brains were removed and stored in 10% formalin for at least 5 days. Frozen sections (40 μm) were obtained using a microtome, and were then placed in a slide for further evaluation. Cannulae placement was verified under a light microscope.

Statistics

Statistical comparisons were performed using analysis of variance (ANOVA) with pretreatment and treatment as independent variables. Dependent variables were the percentages of entries and time spent on the open arms, and the frequency of enclosed arm entries. Multiple post hoc comparisons were performed using the Tukey's HSD test. The level of statistical significance adopted was $p < 0.05$.

RESULTS

In all experiments, the administration of 1.2 g/kg ethanol produced the expected increase in the exploration of the open arms of the elevated plus-maze, without any change in the frequency of enclosed-arm entries, suggesting an anxiolytic effect for this drug (Fig. 1). The results of the influence of pretreatment with dexamethasone 15 min before ethanol in the plus-maze test are shown in Fig. 1. The overall ANOVA for the % open-arm entries revealed a significant effect of the pretreat-

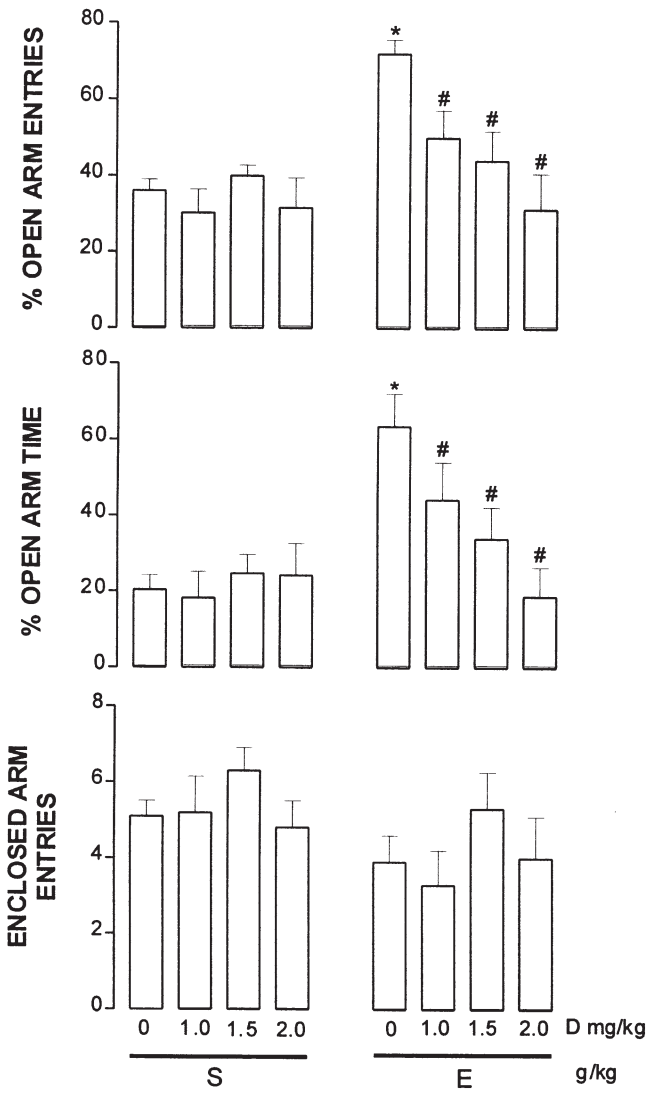

FIG. 1. Influence of pretreatment with dexamethasone (D) on the anxiolytic effect induced by ethanol (E) in rats. Dexamethasone (0.5, 1.0, or 2.0 mg/kg, IP) was injected 15 min before ethanol (1.2 g/kg, 14% w/v, IP), and 15 min later the animals were tested on the elevated plus-maze. S = saline. Each value represents the means ± SEM of 10 animals. *Significantly different from the control group; #significantly different from saline (0) + ethanol (E) group (Tukey's HSD test).

ment, $F(3, 72) = 4.16$, $p < 0.01$, of the ethanol treatment, $F(3, 72) = 10.68$, $p < 0.01$, and of the interaction factor, $F(3, 72) = 3.26$, $p < 0.05$. The ANOVA for the % of time spent on open arms showed a significant main effect of ethanol treatment, $F(3, 72) = 11.45$, $p < 0.01$, and for the interaction factor, $F(3, 72) = 3.80$, $p < 0.05$, without significance for the pretreatment factor, $F(3, 72) = 2.45$, $p = 0.07$. Further comparisons showed that dexamethasone significantly reduced the values of entries and time spent on open arms (Tukey test, $p < 0.05$). ANOVA of enclosed-arm data showed that drug pretreatment produced neither significant effects, $F(3, 72) = 1.49$, $p = 0.22$, nor a significant interaction factor, $F(3, 72) = 0.16$, $p = 0.91$, but produced a significant effect for the treatment factor, $F(1, 72) = 4.36$, $p = 0.04$. Post hoc comparisons did not detect any differences between groups.

Figure 2 depicts the influence of dexamethasone injected 4 h before ethanol treatment. ANOVA for the % open-arm entries revealed significant differences for ethanol treatment, $F(1, 72) = 4.27$, $p = 0.04$, without differences for dexamethasone pretreatment, $F(3, 72) = 0.77$, $p = 0.51$, or interaction factor, $F(3, 72) = 2.55$, $p = 0.06$. Post hoc analysis showed differences between ethanol and saline-treated rats ($p < 0.05$; Tukey test). ANOVA for the % time spent on open arms showed significant differences for ethanol treatment, $F(1, 72) = 7.78$, $p < 0.01$, without differences for dexamethasone pretreatment, $F(3, 72) = 0.01$, $p = 0.99$, or interaction factor, $F(3, 72) = 2.41$, $p = 0.07$. Further post hoc analysis revealed significant differences between saline and ethanol-treated rats ($p <$

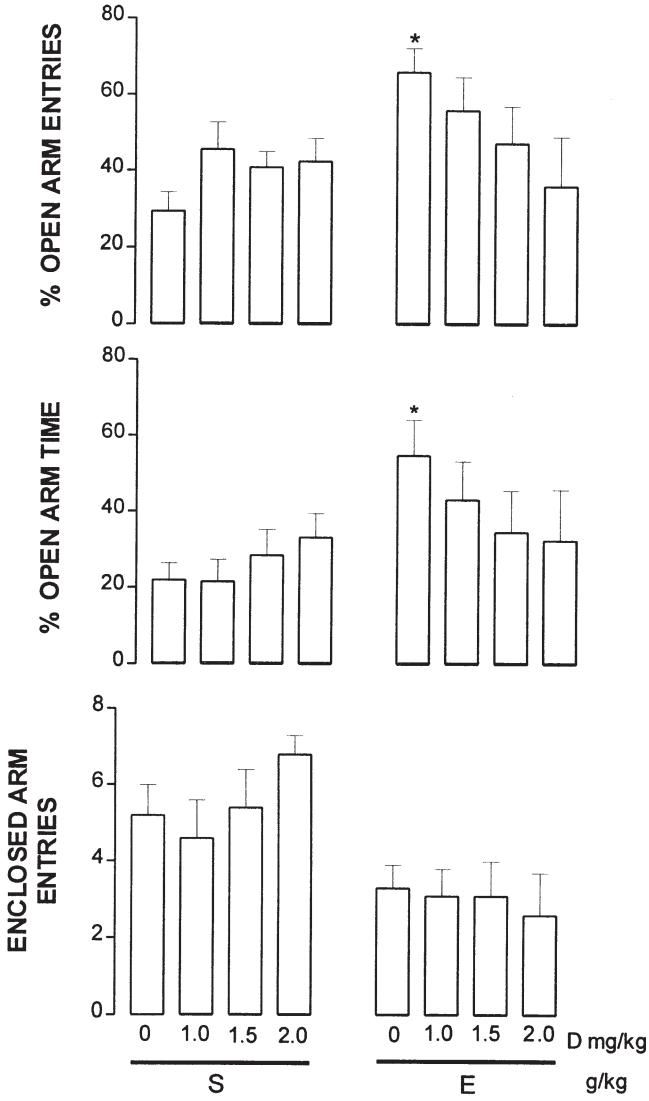

FIG. 2. Influence of pretreatment with dexamethasone (D) on the anxiolytic effect induced by ethanol (E) in rats. Dexamethasone (0.5, 1.0, or 2.0 mg/kg, IP) was injected 4 h before ethanol (1.2 g/kg, 14% w/v, IP), and 15 min later the animals were tested on the elevated plus-maze. S = saline. Each value represents the means ± SEM of 10 animals. *Significantly different from the control group (Tukey's HSD test).

0.05; Tukey test). These results suggest that dexamethasone administered 4 h before ethanol treatment is ineffective.

The effects of intrahippocampal dexamethasone (2 and 20 nmol) on ethanol-induced anxiolysis are presented in Fig. 3. For dexamethasone, 2 nmol separate ANOVAs for the % entries on open arms revealed a significant interaction factor, $F(1, 36) = 9.97$, $p < 0.01$, with nonsignificant effect of pretreatment, $F(1, 36) = 1.59$, $p = 0.21$, and treatment, $F(1, 36) = 2.43$, $p = 0.12$, while for the % time spent on the open arms all factors were significant: pretreatment, $F(1, 36) = 5.93$, $p < 0.05$; treatment, $F(1, 36) = 7.02$, $p < 0.05$, and interaction factor, $F(1, 36) = 15.22$, $p < 0.001$. For dexamethasone 20 nmol data, ANOVA showed a significant interaction factor for the % open-arm entries, $F(1, 36) = 7.17$, $p < 0.05$, and for the % open-arm time, $F(1, 36) = 10.00$, $p < 0.01$ (Fig. 3). Post hoc analysis confirmed that dexamethasone (2 and 20 nmol) significantly reduced the ethanol-induced anxiolysis (Tukey test, $p < 0.05$). Again, no significant differences were detected in the frequencies of enclosed-arm entries: pretreatment, $F(1, 36) = 2.10$, $p = 0.16$; treatment, $F(1, 36) = 1.69$, $p = 0.20$; interaction factor, $F(1, 36) = 3.53$, $p = 0.07$.

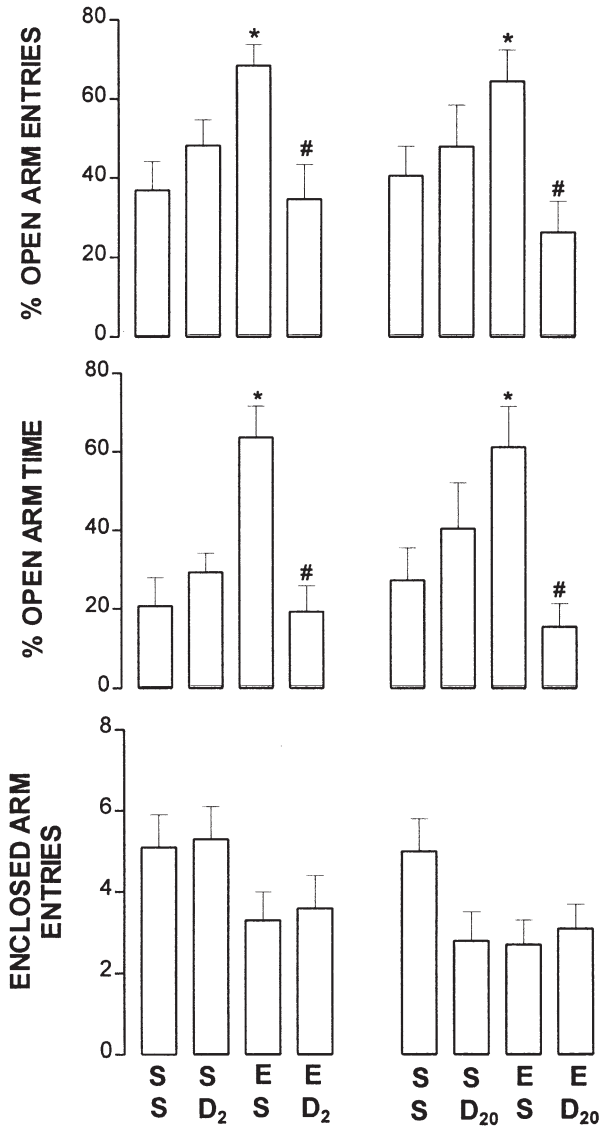

FIG. 3. Influence of treatment with dexamethasone (D) on the anxiolytic effect induced by ethanol (E) in rats. Dexamethasone (2.0 or 20.0 nmol) was injected 10 min after ethanol (1.2 g/kg, 14% w/v, IP), and 5 min later the animals were tested on the elevated plus-maze. S = Saline-treated animals. Each value represents the means ± SEM of 10 animals. *Significantly different from the respective control group; #significantly different from saline (S) + ethanol (E) group (Tukey's HSD test).

DISCUSSION

The present results demonstrate for the first time a clear antagonism of the ethanol-induced anxiolytic effect by systemic and intrahippocampal injections of dexamethasone, a specific glucocorticoid receptor agonist, in rats tested in the elevated plus-maze. Blockade of this anxiolytic response was evident at 15 min but not at 4 h following IP administration of dexamethasone. Also, a rapid antagonism of ethanol-induced anxiolysis was seen after the microinjection of GR agonist into the hippocampus, suggesting that HPA–hippocampal interactions are important modulators of anxiety.

These findings are in agreement with our previous data obtained on the antagonism of ethanol-induced hypothermia by dexamethasone in rats (4) and on the reversion of ethanol-induced hypnosis by dexamethasone and cortisol in mice (34). It is important to note that the doses of glucocorticoid used herein were about 10 times lower than those used in studies of the antagonism of hypnosis (34), but were within the same range used to reverse ethanol-induced hypothermia (2 mg/kg, IP) (4). Moreover, the antagonism of the anxiolytic effect was obtained at doses where dexamethasone per se did not alter anxiety responses in the plus-maze.

Although increased or decreased exploration of the open arms are usually considered as an index of anxiety, measurement of general activity in the elevated plus-maze is still under debate. As discussed by File (12), the frequency of enclosed-arm entries is an important measurement of general activity in this test, appropriate for assessing whether the effect of the drug is an anxiolytic one. In our study, the frequency of enclosed-arm entries was not affected by the treatment with either ethanol or dexamethasone, suggesting that the interaction between these drugs was not a consequence of nonspecific effect on locomotor activity.

Mineralocorticoid- and glucocorticoid-receptor antagonists seem to reduce behavioral measurements related to anxiety in different tests (1,18). Rats preexposed to stressful stimuli showed reduced anxiety-related behaviors in the elevated plus maze after intracerebroventricular administration of MR antagonist (18). Moreover, intrahippocampal pretreatment with a mineralocorticoid antagonist produced anxiolytic effect in rats tested on the plus-maze (1) and black-white box (29). Interestingly, these effects were observed after the short period of time that had elapsed between the injections and the test, suggesting a nongenomic effect (29).

Systemic administration of dexamethasone is known to be a good GR agonist in comparison with MR stimulation in vivo (30), thus activating the inhibition of the HPA axis and thereby decreasing corticosteroid secretions. In the present study, we measured the circulating levels of neither corticosteroid nor ethanol. Although the mechanism underlying the antagonism of ethanol-induced anxiolysis following dexamethasone pretreatment remains unsolved, this response does not seem to be a consequence of an altered ethanol metabolism. For example,

in the study reporting antagonism of hypnosis, the pretreatment with dexamethasone did not affect blood ethanol concentrations (34). Moreover, in our study, intrahippocampal injections of dexamethasone were equally effective in blocking the increased exploration of the open arms in the elevated plus-maze produced by previous injections of ethanol. Therefore, our data suggest that a pharmacodynamic rather than a pharmacokinetic interaction between ethanol and dexamethasone is responsible for the antagonism observed in this study.

The blockade of the ethanol anxiolysis by dexamethasone could be a consequence of an action in both GR or MR, because this glucocorticoid may act in both types of receptor (14). However, because the blockade of ethanol anxiolysis occurred in a short period of time, this fact suggests a direct action at the level of plasma membrane. For example, dexamethasone may alter neurotransmitter levels in the rat central nervous system. Systemic administration of this drug resulted in increased glutamate concentrations in the hippocampus, without affecting aspartate, glycine, GABA, or acetylcholine levels (27). Moreover, the glucocorticoids corticosterone and dexamethasone induced rapid and transient increase in extracellular aspartate and glutamate levels in the CA1 area of the hippocampus obtained through microdialysis in rats. This effect was not blocked by corticosteroid receptor antagonists or by pretreatment with the protein synthesis inhibitor anisomycin, implying a nongenomic mechanism of action in this process (37). On the other hand, it was shown that IP dexamethasone increased, in a time dependent manner, *N*-methyl-D-aspartate–induced excitotoxicity in 7-day-old rats (31). Therefore, it is speculated that the actions of dexamethasone observed in this study might be explainable in terms of interference with neurotransmitter systems, particularly the glutamatergic system, which is also affected by ethanol (36).

In conclusion, the rapid antagonism of the anxiolytic effect of ethanol by dexamethasone pretreatment supports and provides additional evidence for the influence of glucocorticoids in the hippocampus on the modulation of anxiety responses. Certainly, further experiments using protein synthesis inhibitors or selective glucocorticoid receptors antagonists should be conducted to better understand this antagonism.

REFERENCES

1. Bitran, D.; Shiekh, M.; Dowd, J. A.; Dugan, M. M.; Renda, P.: Corticosterone is permissive to the anxiolytic effect that results from the blockade of hippocampal mineralocorticoid receptors. Pharmacol. Biochem. Behav. 60:879–887; 1998.
2. Blanchard, R. J.; Magee, L.; Veniegas, R.; Blanchard, C.: Alcohol and anxiety: Ethopharmacological approaches. Prog. Neuropsychopharmacol. Biol. Psychiatry 17:171–182; 1993.
3. Blatt, S. L.; Takahashi, R. N.: Experimental anxiety and the reinforcing effects of ethanol in rats. Braz. J. Med. Biol. Res. 32:457–461; 1999.
4. Carreño, C. F. T.; Ferreira, V. M. M.; Morato, G. S.: Ethanol-induced hypothermia in rats is antagonized by dexamethasone. Braz. J. Med. Biol. Res. 30:69–72; 1997.
5. Coop, C. F.; Mcnaughton, N.; Warnock, K.; Laverty, R.: Effects of ethanol and Ro 15-4513 in an electrophysiological model of anxiolytic action. Neuroscience 35:669–674; 1990.
6. Dudek, B. C.; Maio, A.; Phillips, T. J.; Perrone, M.: Naturalistic behavioral assessment of anxiolytic properties of benzodiazepines and ethanol in mice. Neurosci. Lett. 63:265–270; 1986.
7. Erickson, C. K.; Graham, D. T.: Alterations in cortical and reticular acetylcholine release by ethanol in vivo. J. Pharmacol. Exp. Ther. 185:583–593; 1973.
8. Fahlke, C.; Engel, J. A.; Eriksson, C. J. P.; Hard, E.; Soderpalm, B.: Involvement of corticosterone in the modulation of ethanol consumption in the rat. Alcohol 3:195–202; 1994.
9. Ferreira, V. M. M.; Morato, G. S.: Influence of age and of pretreatment with D-cycloserine on the behavior of ethanol-treated rats tested in the elevated plus-maze apparatus. Addict. Biol. 1:395–404; 1996.
10. Ferreira, V. M. M.; Morato, G. S.: D-Cycloserine blocks the effects of ethanol and HA-966 in rats tested in the elevated plus-maze. Alcohol. Clin. Exp. Res. 21:1638–1642; 1997.
11. File, S. E.: Chronic exposure to noise modifies the anxiogenic response, but not the hypoactivity, detected on withdrawal from chronic ethanol treatment. Psychopharmacology (Berlin) 116: 369–372; 1994.
12. File, S. E.: Behavioral detection of anxiolytic action. In: Elliott, J. M.; Heal, D. J.; Marsden, C. A., eds. Experimental approaches to anxiety and depression. New York: John Wiley & Sons Ltd.; 1992:25–44.
13. Goldstein, D. B.: Physical dependence on ethanol: Its relation to tolerance. Drug Alcohol Depend. 4:33–42; 1979.
14. Grotjohann, I.; Schulzke, J. D.; Fromm, M.: Electrogenic Na^+ transport in rat late distal colon by natural and synthetic glucocorticosteroids. Am. J. Physiol. 276:491–498; 1999.
15. Imhof, J. T.; Coelho, Z. M. I.; Schmitt, M. L.; Morato, G. S.; Carobrez, A. P.: Influence of gender and age on performance of rats in the elevated plus-maze apparatus. Behav. Brain Res. 56:177–180; 1993.
16. Kiianmaa, K.; Tabakoff, B.: Neurochemical correlates of tolerance and strain differences in the neurochemical effects of ethanol. Pharmacol. Biochem. Behav. 18:383–388; 1983.
17. Korpi, E. R.: Role of $GABA_A$ receptors in the actions of alcohol and in alcoholism: Recent advances. Alcohol Alcohol. 29:115–129; 1994.
18. Korte, S. M.; De Boer, S. F.; De Kloet, E. R.; Bohus, B.: Anxiolytic-like effects of selective mineralocorticoid and glucocorticoid antagonists on fear-enhanced behavior in the elevated plus-maze. Psychoneuroendocrinology 20:385–394; 1995.
19. Korte, S. M.; Korte-Bouws, G. A.; Koob, G. F.; De Kloet, E. R.; Bohus, B.: Mineralocorticoid and glucocorticoid receptor antagonists in animal models of anxiety. Pharmacol. Biochem. Behav. 54:261–267; 1996.
20. Lepola, U.: Alcohol and depression in panic disorder. Acta Psychiatr. Scand. 377:33–35; 1994.
21. Montgomery, K. C.: The relation between fear induced by novel stimulation and exploratory behaviour. J. Comp. Physiol. Psychol. 48:254–260; 1955.
22. Nevo, I.; Hamon, M.: Neurotransmitter and neuromodulatory mechanisms involved in alcohol abuse and alcoholism. Neurochem. Int. 26:305–336; 1995.
23. Polivy, J.; Schueneman, A. L.; Carlson, K.: Alcohol and tension reduction: Cognitive and physiological effects. J. Abnorm. Psychol. 85:595–600; 1976.
24. Pruett, S. B.; Collier, S. D.; Wu, W. J.: Ethanol-induced activation of the hypothalamic–pituitary–adrenal axis in a mouse model for binge drinking: Role of Ro 15-4513-sensitive gamma aminobutyric acid receptors, tolerance, and relevance to humans. Life Sci. 63:1137–1146; 1998.
25. Randin, D.; Vollenweider, P.; Tappy, L.; Jéquier, E.; Nicod, P.; Scherrer, U.: Suppression of alcohol-induced hypertension by dexamethasone. N. Engl. J. Med. 332:1733–1737; 1995.
26. Roberts, A. J.; Crabbe, J. C.; Keith, L. D.: Corticosterone increases severity of acute withdrawal from ethanol, pentobarbital, and diazepam in mice. Psychopharmacology (Berlin) 115:278–284; 1994.
27. Semba, J.; Miyoshi, R.; Kanazawa, M.; Kito, S.: Effect of systemic dexamethasone on extracellular amino acids, GABA and acetylcholine release in rat hippocampus using in vivo microdialysis. Funct. Neurol. 10:17–21; 1996.

28. Shefner, A. S.; Tabakoff, B.: Basal firing rate of rat locus coeruleus neurons affects sensitivity to ethanol. Alcohol 2:239–243; 1985.
29. Smythe, J. W.; Murphy, D.; Timothy, C.; Costall, B.: Hippocampal mineralocorticoid, but not glucocorticoid, receptors modulate anxiety-like behavior in rats. Pharmacol. Biochem. Behav. 56:507–513; 1997.
30. Spencer, R. L.; Miller, A. H.; Stein, M.; McEwen, B. S.: Corticosterone regulation of type I and type II adrenal steroid receptors in brain, pituitary and immune tissue. Brain Res. 549:236–246; 1991.
31. Supko, D. E.; Johnston, M. V.: Dexamethasone potentiates NMDA receptor-mediated neuronal injury in the postnatal rat. Eur. J. Pharmacol. 270:105–113; 1994.
32. Sze, P. Y.: The permissive role of glucocorticoids in the development of ethanol dependence and tolerance. Drug Alcohol Depend. 2:381–396; 1977.
33. Sze, P. Y.; Feigenbaum, J. J.; Yanai, J.: Adrenal glucocorticoids as a required factor in the development of ethanol tolerance in mice. Neuropsychobiology 9:207–210; 1983.
34. Sze, P. Y.: Glucocorticoids antagonize the sedative action of ethanol in mice. Pharmacol. Biochem. Behav. 45:991–993; 1993.
35. Tabakoff, B.; Hoffman, P. L.; Moses, F.: Neurochemical correlates of ethanol withdrawal: Alterations in serotonergic function. J. Pharm. Pharmacol. 29:471–476; 1977.
36. Tsai, G.; Coyle, J. T.: The role of glutamatergic neurotransmission in the pathophysiology of alcoholism. Annu. Rev. Med. 49:173–184; 1998.
37. Venero, C.; Borrell, J.: Rapid glucocorticoid effects on excitatory amino acid levels in the hippocampus: A microdialysis study in freely moving rats. Eur. J. Neurosci. 11:2465–2473; 1999.
38. Wang, X.; Lemos, J. R.; Dayanithi, G.; Nordmann, J. J.; Treistman, S. N.: Ethanol reduces vasopressin release by inhibiting calcium currents in nerve terminals. Brain Res. 551:338–341; 1991.
39. Wilson, G. T.; Abrams, D. B.: Effects of alcohol on social anxiety and physiological arousal: Cognitive versus pharmacological processes. Cog. Ther. Res. 1:195–210; 1977.