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Reduction of Flurazepam's Antiseizure Efficacy Persists After Stress

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MASTROPAOLO, J., C. H. PARK, D. O. NORRIS, D. A. O'CONNOR, L. G. LUKACS AND S. I. DEUTSCH. *Reduction of flurazepam's antiseizure efficacy persists after stress.* PHARMACOL BIOCHEM BEHAV **42**(4) 681-684, 1992. – Twenty-four hours after mice were forced to swim for up to 10 min in cold (6°C) water, the ability of flurazepam to antagonize the electrical precipitation of seizures was reduced. This stress-induced reduction in flurazepam's antiseizure efficacy persisted for at least 72 h; but was absent 1 week after the single session of swim stress. The data may be relevant to stress-related psychiatric disorders and suggest that the therapeutic efficacy of benzodiazepines may be altered after a severe stress.

Stress Flurazepam Seizures

TWENTY-FOUR hours after mice were forced to swim for up to 10 min, the ability of flurazepam to antagonize the electrical precipitation of seizures was reduced (2). The severity of this stress-induced reduction in the antiseizure efficacy of flurazepam was greater in mice forced to swim in cold water (6°C) compared with animals forced to swim in water at ambient temperature (24°C). Thus, it may be that the greater the severity of the stressor the greater the reduction in flurazepam's antiseizure efficacy. There are data suggesting that this stress-induced reduction of flurazepam's antiseizure efficacy may result from a decreased density of benzodiazepine binding sites in the cerebral cortex, hippocampus, and hypothalamus (10).

Moreover, an intact hypothalamic-pituitary-adrenal axis may be necessary for stress-induced changes in benzodiazepine receptor binding. Adrenalectomy prevented swim stress-induced reductions in the binding of [^{3}H]Ro 15-1788, as shown with an in vivo approach to measuring benzodiazepine receptor binding (10). Adrenal steroids are released during stress and they, as well as A-ring-reduced metabolites of progesterone and deoxycorticosterone, may influence the functioning of the $GABA_A$ receptor complex. This could occur through delayed effects on gene regulation and expression of specific polypeptides, as well as direct effects on the $GABA_A$ receptor complex itself. For example, it is known that the A-ring-reduced metabolites of progesterone and deoxycorticosterone act as barbiturate-like ligands capable of promoting chloride ion conductance (6). Moreover, forced swim stress may not affect all populations of central benzodiazepine binding sites in an identical manner; swim stress did not significantly alter flurazepam's effect on mouse rotorod performance (2). Conceivably, the population of central benzodiazepine binding sites mediating the sedative-ataxic properties of flurazepam may be less sensitive to stress than the population mediating its ability to antagonize the electrical precipitation of seizures.

The current investigation examined the persistence of this stress-induced reduction in flurazepam's antiseizure efficacy following a single session of cold water swim stress. Specifically, flurazepam's ability to antagonize the electrical precipitation of seizures was studied 24, 48, and 72 h and 1 week after forcing mice to swim for up to 10 min in cold water (6°C).

METHOD

Subjects

Experimentally naive, male NIH Swiss mice weighing approximately 30 g were used throughout the experiments.

Drugs

Flurazepam was generously donated by Hoffmann-La Roche (Nutley, NJ). Flurazepam was dissolved in distilled,

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deionized water approximately 2 h prior to the incremental electroconvulsive shock (IECS) procedure. Flurazepam and its vehicle were injected intraperitoneally (IP) in a volume of 0.01 ml/g body weight.

IECS Procedure

In the IECS procedure, a Hittman electroconvulsive shock generator (Medcraft model B24-III, Skippack, PA) was utilized to administer 0.3 s of voltage via earclip electrodes. Except where indicated, the procedure began with 70 V and was increased in 10-V increments every 2 s until a full seizure (maximal tonic hindlimb extension) occurred or 170 V was reached. This procedure was approved by the Animal Studies Subcommittee and Research Committee of this Department of Veterans Affairs Medical Center. The antiseizure efficacy of flurazepam was assessed in groups of mice that received injections of either vehicle or increasing doses of flurazepam 20 min prior to the IECS procedure. In all experiments, groups of at least 12 mice were tested in each of the experimental conditions.

Stress Procedure

Mice were forced to swim in cold water (6°C) for up to 10 min 24, 48, and 72 h and 1 week prior to testing with the IECS procedure.

Data Analysis

Data from each experiment were subjected to a between-subjects two-way analysis of variance (ANOVA) using flurazepam dose and stress as between-subjects factors. An overall analysis of the data was not done because of the variability inherent in the flurazepam dose-response curve replications. For the purpose of data analysis, animals that did not seize were assigned a voltage of 180.

RESULTS

As shown in Fig. 1, the mean threshold convulsant voltage of stressed animals did not differ from that of unstressed control mice. Specifically, in these experiments the IECS procedures began with 17.5 and 35 V and the voltages were increased in increments of 5 V. Stressed mice and unstressed controls did not differ from each other with respect to their mean threshold convulsant voltage; both groups seized at a mean threshold voltage of approximately 55 V. These data extend our original observation that the mean threshold convulsant voltage is similar in vehicle-treated animals irrespective of prior stress history. Thus, stress-induced changes in mean threshold convulsant voltages only become apparent in mice treated with flurazepam.

As shown in Fig. 2, consistent with the results of our previous experiments, flurazepam significantly increased the mean threshold convulsant voltage in all groups and its effectiveness was significantly reduced in stressed animals. A two-way ANOVA revealed significant main effects for stress ($p = 0.0004$) and flurazepam dose ($p = 0.0001$).

Figures 3 and 4 show that flurazepam's diminished antiseizure efficacy persisted for 48 and 72 h after a single 10-min session of cold water swim stress. A two-way ANOVA showed a main effect for stress at 48 h ($p < 0.08$) and a significant main effect for stress at 72 h ($p < 0.01$) after the swim stress. Again, vehicle-treated stressed and control mice did not differ

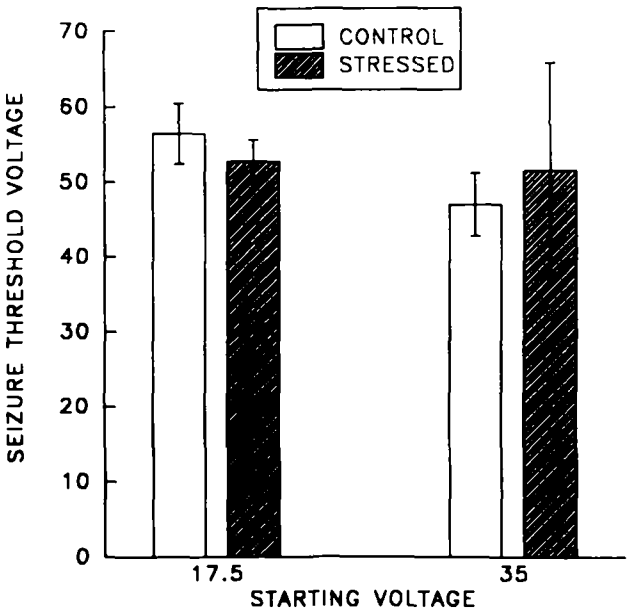

FIG. 1. Mean threshold convulsant voltages of stressed mice and control mice did not differ. In these experiments, the IECS procedures began with 17.5 and 35 V and the voltages were increased in increments of 5 V.

from each other with respect to the mean threshold voltage for seizure production.

Figure 5 shows that flurazepam's antiseizure efficacy did not differ between stressed and control animals 1 week after a single session of cold water swim stress ($p = 0.39$).

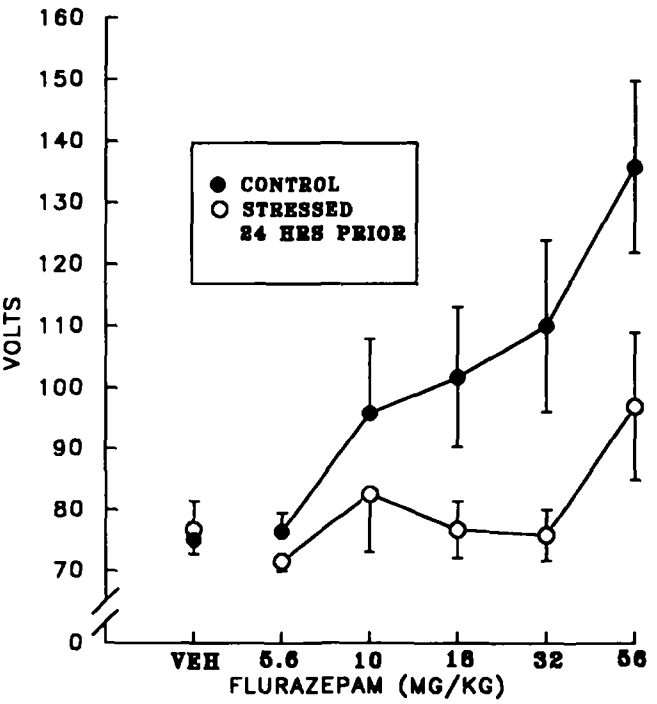

FIG. 2. Mean threshold convulsant voltages of stressed mice and control mice given vehicle or various doses of flurazepam 24 h after a single session of cold water swim stress.

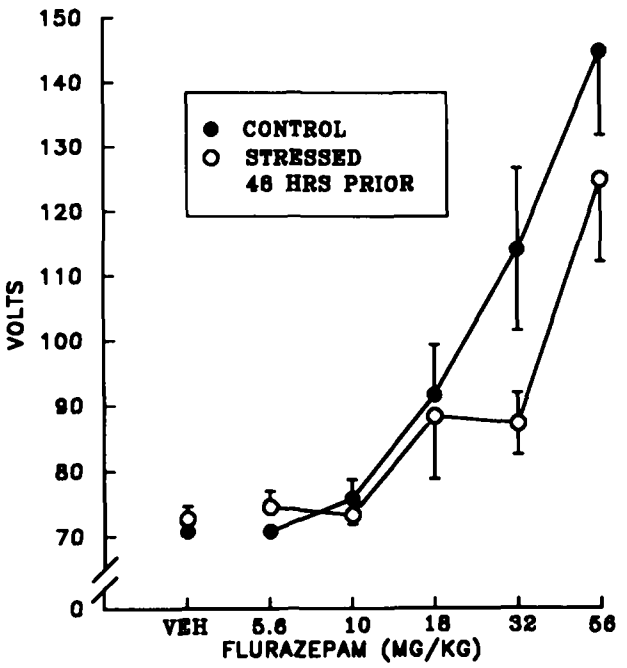

FIG. 3. Mean threshold convulsant voltages of stressed mice and control mice given vehicle or various doses of flurazepam 48 h after a single session of cold water swim stress.

DISCUSSION

In this study, a single session of profound and inescapable stress was shown to have an enduring effect on benzodiazepine receptor sensitivity as reflected in a diminished antiseizure efficacy of flurazepam for up to 3 days after the stressful event. The single session of stress did not alter the threshold voltage for seizure production in vehicle-treated animals; the effect of stress was only apparent in animals treated with flurazepam 20 min prior to the IECS procedure. Thus, the effect of swim stress was not a nonspecific one on seizure threshold per se. Prior investigations showed that stress-induced alterations in the functioning of the $GABA_A$ receptor complex can occur rapidly (i.e., within 1 min or less after stress) (4,5,9). The mechanism of the rapid stress-induced changes in $GABA_A$ receptor functioning is unknown but could involve either posttranslational modification of the receptor, release of an endogenous benzodiazepine-like ligand, or a combination of the two (3). A unique intracellular site for the cAMP-dependent phosphorylation of a serine residue has been described on the β-subunit (8). This phosphorylation site could serve as the substrate for rapid posttranslational modification of the $GABA_A$ receptor complex. Moreover, an endogenous β-carboline has been detected in the cerebral cortex of acutely stressed rats (7). The persistence of altered $GABA_A$ receptor functioning following stress could involve altered expression of the amounts of individual polypeptide chains that contribute to functional ligand-gated channels. For example, it is conceivable that stress could reduce expression of polypeptides that influence the binding of benzodiazepines (e.g., the γ_2 subunit) in selected regions of the brain (1,11). If this was so, then the number of functional $GABA_A$ receptors whose ligand-stimulated chloride ion conductance could be modified by benzodiazepines would be reduced. Moreover, an actual reduction in the synthesis of polypeptides capable of binding benzodiazepines would be reflected in a reduction in the maximal density of specific binding sites for [^{3}H]flunitrazepam. The data also suggest that the behavioral measure we employed (i.e., flurazepam's ability to antagonize the electrical precipitation of seizures in the IECS procedure) may reflect functional changes in the $GABA_A$ receptor complex.

The results of this study may be relevant to the pathophysi-

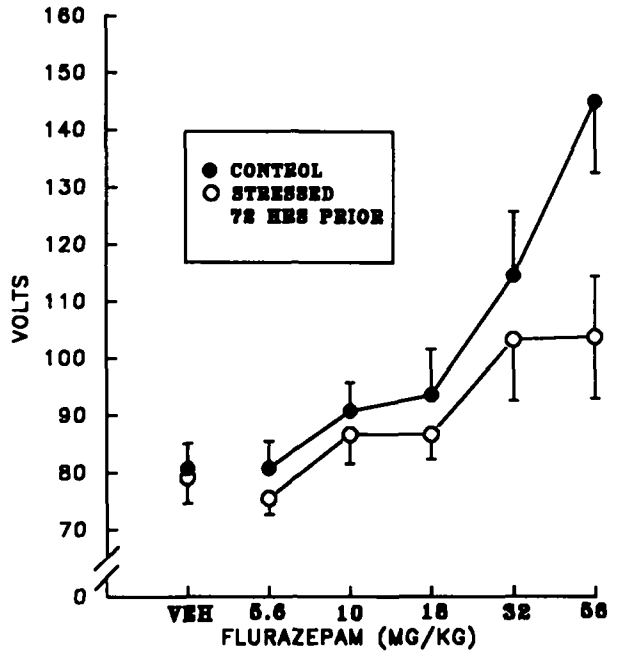

FIG. 4. Mean threshold convulsant voltages of stressed mice and control mice given vehicle or various doses of flurazepam 72 h after a single session of cold water swim stress.

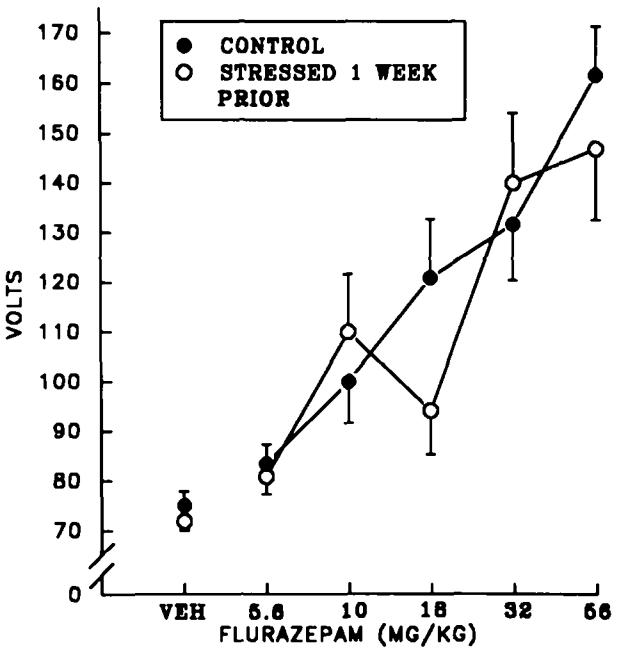

FIG. 5. Mean threshold convulsant voltages of stressed mice and control mice given vehicle or various doses of flurazepam 1 week after a single session of cold water swim stress.

ology of stress-related psychiatric disorders. Also, the results suggest that the efficacy of benzodiazepines may be altered in patients for at least several days after a catastrophic stressful event. In any event, the major observation of this study is that a single exposure to a profound stress in an adult animal can have enduring effects on the ability of a prototypic benzodiazepine to antagonize the electrical precipitation of seizures. Future studies will examine the enduring impact, if any, of stress on benzodiazepine receptor functioning in mouse pups. Also, we are interested in knowing whether some of these stress-induced alterations can be prevented. In a previous report, treatment with Ro 15-1788, a relatively specific benzodiazepine antagonist, prior to stress attenuated the reduction in flurazepam's antiseizure efficacy (2).

REFERENCES

1. Barnard, E. A.; Darlison, M. G.; Fujita, N.; Glencorse, T. A.; Levitan, E. S.; Reale, V.; Schofield, P. R.; Seeburg, P. H.; Squire, M. D.; Stephenson, F. A. Molecular biology of the $GABA_A$ receptor. Adv. Exp. Med. Biol. 236:31-45; 1988.
2. Deutsch, S. I.; Rosse, R. B.; Huntzinger, J. A.; Novitzki, M. R.; Mastropaolo, J. Profound stress-induced alterations in flurazepam's antiseizure efficacy can be attenuated. Brain Res. 520:272-276; 1990.
3. Deutsch, S. I.; Weizman, A.; Weizman, R.; Vocci, F. J.; Kook, K. A. Role of the GABA-benzodiazepine receptor complex in stress. In: Deutsch, S. I.; Weizman, A.; Weizman, R., eds. Application of basic neuroscience to child psychiatry. New York: Plenum; 1990:61-76.
4. Havoundjian, H.; Paul, S. M.; Skolnick, P. Rapid, stress-induced modification of the benzodiazepine receptor-coupled chloride ionophore. Brain Res. 375:401-406; 1986.
5. Havoundjian, H.; Paul, S. M.; Skolnick, P. Acute, stress-induced changes in the benzodiazepine/GABA receptor complex are confined to the chloride ionophore. J. Pharmacol. Exp. Ther. 237:787-793; 1986.
6. Majewska, M. D.; Harrison, N. L.; Schwartz, R. D.; Barker, J. L.; Paul, S. M. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 232:1004-1007; 1986.
7. Novas, M. L.; Wolfman, C.; Medina, J. H.; De Robertis, E. Proconvulsant and "anxiogenic" effects on *n*-butyl-*B*-carboline-3-carboxylate, an endogenous benzodiazepine inhibitor from brain. Pharmacol. Biochem. Behav. 30:331-336; 1988.
8. Schofield, P. R.; Darlison, M. G.; Fujita, N.; Burl, D. R.; Stephenson, F. A.; Rodriguez, H.; Rhee, L. M.; Ramachandran, J.; Reale, V.; Glencorse, T. A.; Seeburg, P. H.; Barnard, E. A. Sequence and functional expression of the $GABA_A$ receptor shows a ligand-gated receptor super-family. Nature 328:221-227; 1987.
9. Trullas, R.; Havoundjian, H.; Zamir, N.; Paul, S. M.; Skolnick, P. Environmentally-induced modification of the benzodiazepine/GABA receptor coupled chloride ionophore. Psychopharmacology (Berl.) 91:380-384; 1987.
10. Weizman, A.; Weizman, R.; Kook, K. A.; Vocci, F.; Deutsch, S. I.; Paul, S. M. Adrenalectomy prevents the stress-induced decrease in *in vivo* [^{3}H]RO15-1788 binding to $GABA_A$ benzodiazepine receptors in the mouse. Brain Res. 519:347-350; 1990.
11. Ymer, S.; Draguhn, A.; Wisden, W.; Werner, P.; Keinanen, K.; Schofield, P. R.; Sprengle, R.; Pritchett, D. B.; Seeburg, P. H. Structural and functional characterization of the $gamma_1$ subunit of $GABA_A$/benzodiazepine receptors. EMBO J. 9(10):3261-3267; 1990.