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RAPID COMMUNICATION

Paradoxical Effect of Flurazepam

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DEUTSCH, S. I., D. O. NORRIS, D. A. O'CONNOR AND J. MASTROPAOLO. *Paradoxical effect of flurazepam.* PHARMACOL BIOCHEM BEHAV **42**(3) 517–518, 1992.—Cold water swim stress has been shown to decrease the ability of flurazepam, a prototypic GABA-positive benzodiazepine, to antagonize the electrical precipitation of seizures in mice. This stress-induced reduction in the antiseizure efficacy of flurazepam is not due to a reduction in the threshold voltage for seizure production. In this study, we examined the effect of treating mice with flurazepam 20 min prior to cold water swim stress on its ability to antagonize electrically precipitated seizures 24 h later. Contrary to our expectation, pretreatment with flurazepam potentiated the stress-induced reduction of its antiseizure efficacy.

Stress Benzodiazepine receptor Flurazepam Seizures

BENZODIAZEPINES bind with high affinity and stereoselectivity to a site that is a component of the $GABA_A$ receptor complex (3,4). The properties of this binding site are determined by the association of the individual polypeptide subunits comprising the $GABA_A$ receptor complex (5).

A 10-min session of cold water swim stress has been shown to reduce the ability of flurazepam, a prototypic GABA-positive benzodiazepine, to antagonize the electrical precipitation of seizures in mice (1). This stress-induced reduction in flurazepam's antiseizure efficacy persisted for up to 3 days after the single session of cold water swim stress (2). Moreover, the threshold voltage for seizure production was not different in stressed mice and controls; differences emerged only in the presence of flurazepam (2).

The anxiolytic action of GABA-positive benzodiazepines are expected to attenuate physiologic and subjective effects of stress. Therefore, we studied the effect of treating mice with flurazepam prior to a session of cold water swim stress on its antiseizure efficacy when tested 24 h later in the incremental electroconvulsive shock (IECS) paradigm. Contrary to our expectation, flurazepam pretreatment potentiated the stress-induced reduction in its antiseizure efficacy.

METHOD

In this experiment, naive NIH Swiss mice (25–30 g) were divided into four groups (72/group). On day 1, two of the groups were injected with flurazepam (18 mg/kg, IP) and the other two with distilled water (0.01 ml/g). Twenty minutes later, one flurazepam- and one water-injected group were forced to swim for up to 10 min in cold water (6°C) and the other two remaining groups were returned to their home cages. On day 2, all animals were injected with vehicle or one of several doses of flurazepam (5.6, 10, 18, 32, or 56 mg/kg, IP) 20 min prior to the 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. 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. Data from all four groups were subjected to a three-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Three significant main effects were observed (Fig. 1). Specifically, groups pretreated with flurazepam were significantly different from vehicle-pretreated animals, $F(1, 264) =$

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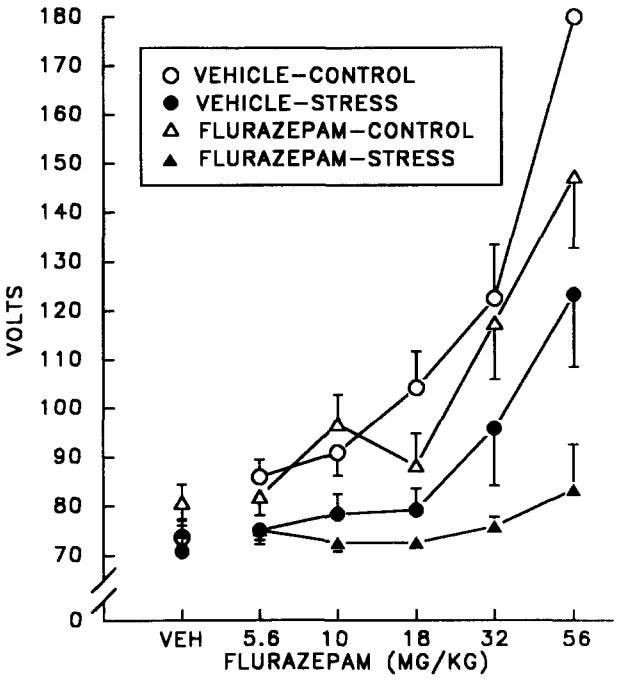

FIG. 1. Mean threshold voltage for the electrical precipitation of seizures in groups of animals pretreated with flurazepam (triangles) or vehicle (circles) 20 min prior to 10 min of cold water swim stress (filled symbols) or no stress manipulation (open symbols) on day 1. The horizontal axis indicates the injection given 20 min prior to the IECS procedure.

11.4, $p = 0.0008$, and stressed animals were significantly different from nonstressed controls, $F(1, 264) = 76.0$, $p < 0.0001$. In addition, there was a significant effect for flurazepam dose on day 2, $F(5, 264) = 40.3$, $p < 0.0001$. Subsequent analyses revealed that in stressed animals those pretreated with flurazepam differed significantly from those pretreated with vehicle, whereas in nonstressed animals those pretreated with flurazepam and vehicle did not differ.

These data are consistent with our previous results showing that stress attenuates the ability of flurazepam to antagonize the electrical precipitation of seizures. It is important to note that flurazepam pretreatment on day 1 had no effect except in the presence of stress. That flurazepam pretreatment in nonstressed animals had no effect is important for two reasons. First, it suggests that active GABA-positive benzodiazepine was cleared from the brain by day 2. Second, it indicates that there was no development of tolerance to the antiseizure effects of flurazepam. In summary, contrary to our expectation, the data show treating animals with a GABA-positive benzodiazepine prior to stress potentiates the stress-induced reduction in flurazepam's antiseizure efficacy. Conceivably, occupation of the central benzodiazepine binding site during stress with a GABA-positive ligand locks the $GABA_A$ receptor complex in a configuration that increases its sensitivity to the effects of this stress.

ACKNOWLEDGEMENTS

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