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Effects of Water Immersion Stress on Convulsions Induced by Pentylenetetrazol

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ABEL, E. L. AND R. F. BERMAN. *Effects of water immersion stress on convulsions induced by pentylenetetrazol.* PHARMACOL BIOCHEM BEHAV **45**(4) 823–825, 1993. – Increasing durations of water immersion stress were associated with increased serum corticosterone levels, decreased severity of pentylenetetrazol-induced convulsions, and increased latencies to convulse in rats. The results were interpreted in terms of the effects of stress and pentylenetetrazol's actions on GABAergic mechanisms.

Stress Convulsions Pentylenetetrazol Rat

ALTHOUGH the influence of stress on susceptibility of animals to convulse has been widely studied, there is considerable inconsistency in the data. Some studies have reported stress enhances seizure susceptibility in rats [e.g., (6,13)], whereas others have found attenuating effects [e.g., (7,14)]. One reason for the inconsistencies in these studies is that the stress factor for rats is often induced by foot-shock [e.g., (10, 13,14,17,21)]. Whereas foot-shock reliably induces a stress response, as reflected in increases in serum corticosterone levels (5), increases in this stress response do not change monotonically with changes in parameters of foot-shock (e.g., intensity, duration) (11). An absence of stressor sensitivity could underline the inconsistencies noted in conjunction with foot-shock and seizure susceptibility. In contrast to foot-shock, recent studies from our laboratory have shown that water immersion is both a reliable and sensitive stressor in rats (1–3). In this regard, De Lima and Rae (7) have reported an increased latency to convulse in pentylenetetrazol-treated mice following a 3-min swim stress. The present study extends that finding by showing that the latency of pentylenetetrazol-induced seizures is directly related to duration of swim stress.

METHOD

Male Sprague-Dawley rats (Charles River, Portage, MI) 60–70 days of age were housed in polycarbonate cages in a vivarium at a room temperature of 21 ± 1°C with a 12L : 12D cycle (lights on at 7:00 a.m.). Food and water were available ad lib. For water immersion stressing, rats were immersed in a Plexiglas cylinder (Corning Glass, NY) (45.7 cm height, 22.2 cm inside diameter) filled to a height of 38 cm with fresh tap water at 27 ± 1°C.

To evaluate the effect of duration of water immersion on stress, animals were immersed for either 10 (N = 10) or 20 min (N = 16). Upon removal from the cylinder, each animal was blotted dry and sacrificed by decapitation. Decapitation occurred in a room adjacent to and separate from the room in which animals were tested. Less than 30 s elapsed from removal of an animal from water and sacrifice. This time interval produces no detectable increase in plasma corticosterone response following maximal ACTH stimulation (19). Control animals (N = 14) were sacrificed immediately after removal from their home cages (time = 0). Trunk blood was collected in tubes and stored on ice for 20 min prior to centrifugation (300 × g for 10 min). The serum was then separated and stored at −70°C until subsequent assay. All sampling was done between 8:30 a.m. and 11:00 a.m.

Serum corticosterone levels were determined using a commercially available radioimmunoassay kit containing corticosterone antibody (ICN Biochemicals). Radioactivity in each sample was counted (Micromedic 4/600 Plus Automatic Gamma counter) and the amount of corticosterone in each sample was determined by comparison to external standards.

To determine the effect of stress on seizure susceptibility, another group of male and female rats was stressed for 0, 10, or 20 min in the inescapable swim test (N = 11–18/time/sex). Animals were removed, blotted dry, and left for 1 min in their cages, after which they were injected (IP) with 45 mg/kg

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pentylenetetrazol (conc: 25 mg/ml in distilled water). This dose was based on a previous dose-response study from our laboratory showing that this dose reliably induced seizures in 80% of all tested animals (4). Animals were scored for occurrence and latency of subclonic, generalized forelimb clonic or tonic convulsions for 10 min (16). Animals that did not seize were assigned latencies of 600 s.

The data for corticosterone and latencies to convulse were analyzed by one-way analysis of variance (ANOVA). Seizure incidence was analyzed by chi-square analysis.

RESULTS AND DISCUSSION

Rats immersed in water attained a mean (±SE) serum corticosterone level of 209 ± 12 ng/ml after 10 min and 318 ± 19 ng/ml after 20 min of immersion compared to 4 ± 1 ng/ml for nontreated controls, $F(2, 37) = 133$, $p < 0.0001$. Thus, increasing durations of water immersion resulted in a proportional increase in serum corticosterone, a widely recognized stress marker (5).

Latency to seize was also significantly increased monotonically with increasing duration of water immersion stress, $F(2, 83) = 3.55$, $p < 0.03$. Neither the gender nor the gender × duration interaction were statistically significant. The effect of stress on seizure latency is shown in Table 1 for both sexes combined.

Chi-square analysis indicated that intensity of seizure was also significantly related to duration of water immersion stress. There were no tonic seizures. Animals experiencing stress prior to injection experienced far fewer clonic convulsions than nonstressed animals, $\chi^2(4) = 26.4$, $p < 0.0001$.

TABLE 1

EFFECTS OF INESCAPABLE SWIM STRESS ON PENTYLENETETRAZOL-INDUCED SEIZURES IN RATS

Duration of Stress (min)	Latency to Seize (Mean ± SE)	Severity		
		No Seizures	Subclonic	Clonic
0 ($N = 35$)	217 ± 36	8	13	14
10 ($N = 24$)	297 ± 42	7	17	0
20 ($N = 30$)	347 ± 39*	12	18	0†

*$F(2, 83) = 3.55, p < 0.03$.
†$\chi^2(4) = 26.4, p < 0.0001$.

Although we did not determine the duration of seizures, stressed animals appeared to have considerably shorter seizures as well.

The increase in serum corticosterone levels following water immersion stress corroborates previous studies from our laboratory (1,3). After experiencing such stress, rats injected with pentylenetetrazol had no clonic seizures and had significantly longer latencies for subclonic seizures than control rats, and these latencies were related to duration of water immersion stress. These results corroborate a similar report by De Lima and Rae (7) for pentylenetetrazol-induced seizures in mice. In that study, only one time period of immersion (3 min) was evaluated. The present study thus extends De Lima and Rae's (7) finding by showing that, a) the longer the duration of immersion, the greater the stress, as reflected in increased corticosteroid levels, and b) the greater the stress, the greater the inhibitory effect on seizure latencies. In another context, Terman et al. (17) have also reported that increasing durations of water immersion stress have an increasing analgesic effect in rats.

The mechanism by which stress influences pentylenetetrazol convulsions is unclear. Several studies have implicated involvement of GABA receptors in stress-related inhibition of seizure thresholds [e.g., (8,16)]. Pentylenetetrazol's mechanism of action is thought to be due to blocking $GABA_A$ receptors (9,20), which, in turn, results in increased neural excitability and epileptiform seizures. In contrast to pentylenetetrazol's effects, stress causes an initial release of GABA at the $GABA_A$ chloride ionophore receptors [e.g., (8,16)]. The decrease in susceptibility to pentylenetetrazol-induced seizures in swim-stressed animals may thus reflect an initial release of GABA at the $GABA_A$ receptors prior to their blockage by pentylenetetrazol, resulting in an overall decrease in pentylenetetrazol's GABAergic convulsant effect.

Alternatively, water immersion may delay the onset of pentylenetetrazol seizure indirectly by altering the activity of other neurochemical systems (e.g., opioid, adrenergic) affected by stress [cf. (7)], or by altering the pharmacokinetics of pentylenetetrazol. For example, changes in blood flow or hypothermia during water immersion could alter the distribution of subcutaneously administered pentylenetetrazol and thereby delay the onset of convulsions. However, De Lima and Rae (7) failed to find significant correlations between hypothermia and latencies to pentylenetetrazol convulsions following swim stress in mice, and the water temperature in the present study (27°C) was much higher than in their study (20°C).

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