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Alarm Substance Induces Convulsions in Imipramine-Treated Rats

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ABEL, E. L., P. J. BILITZKE AND D. B. COTTON. *Alarm substance induces convulsions in imipramine-treated rats.* PHARMACOL BIOCHEM BEHAV **41**(3) 599-601, 1992.—Male rats were injected with imipramine (0-30 mg/kg) and subsequently tested in the forced-swim test in either fresh water or water soiled by other rats, which presumably contains an alarm substance. Imipramine did not affect the behavior of rats in fresh water. More than half the animals given the combination of imipramine (30 mg/kg) and stress from alarm substance had clonic convulsions. Adrenalectomy did not affect this relationship. This is the first study demonstrating the potential of an alarm substance for inducing convulsions.

Convulsions Imipramine Alarm substance Stress Adrenalectomy

ALARM substances, first described in fish in 1938 by Von Frisch (8), are also present in various species including plants (7), insects (31), reptiles (11), and mammals (12,13,22,33,35). In insects and animals, these substances elicit freezing, defensive behavior, dispersal, or aggregation depending on test conditions (13,37).

Recently, exposure to alarm chemosignals has also been found to suppress immune function in rats and mice (14). This interesting phenomenon may be related to pituitary-adrenal (PA) axis activation since stress-related odors cause increases in adrenal activity (14) and PA activation of the pituitary-adrenal axis has long been known to be immunosuppressive (25). In the course of our series of studies on alarm chemosignals in rats (1,3,5), we examined the effects of the antidepressant drug imipramine on the inhibition of immobility produced by alarm chemosignals in the forced-swim test, and made the startling discovery that these chemosignals also have the potential for causing clonic convulsions in rats. The reason we studied imipramine is that the forced-swim test has been regarded as an animal model of depression and the antidepressant imipramine has been used as a prototypic compound in this test (27).

In our paradigm, alarm chemosignals are generated when animals are immersed in a cylinder of water from which there is no escape. The apparatus and test procedure are a variation of the Porsolt et al. (27) forced-swim test. Our variation focuses on testing animals on the first day of immersion (Porsolt tests animals on the second day) in which the water is either fresh or soiled by a previous animal. (The Porsolt test uses only fresh water.) The behavioral difference seen in these two conditions is that animals tested in fresh water initially paddle vigorously but eventually become immobile, whereas those tested in soiled water do not float and continue to paddle vigorously. Since stressing an animal prior to immersion in fresh water also reduces immobility, we interpreted absence of immobility in the rat in this test as reflecting heightened fear or stress (5), an interpretation shared by others [e.g., (10,20,24,27)] but not by all researchers using this procedure (27). Although we have not yet characterized the alarm substance in soiled water that causes rats subsequently tested in it to exhibit alarm-like behavior, we have been able to characterize some of its parameters. Thus far, we have found that it produces a dose-response-like effect on recipients, that is, the greater the concentration the greater the inhibition of immobility (1), it can be detected as long as 8 days after it has been released (1), and it is not readily depleted by repeated testing (1). The substance also satisfies all the criteria for pheromones advanced by Beauchamp et al. (9), viz., it produces behavioral effects in recipients, its effects do not require experience for either emission or detection, it has species specificity, and its behavioral effects are not due to nonspecific arousal (3). We have also shown that this substance has low volatility (3). However, we have not as yet identified any specialized gland that produces or releases this substance such as that which occurs in connection with classic pheromones (33).

The following study was conducted to examine our preliminary observation that the combination of imipramine and stress from alarm chemosignals induces convulsions in rats.

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METHOD

Male Sprague-Dawley rats (Charles River, Portage, MI), 70–80 days of age, were housed in polypropylene cages in a vivarium at a constant room temperature of 21 ± 2°C and humidity (50 ± 5%) and a 12 L:12 D cycle. Food and water were available ad lib. Animals were immersed in a Plexiglas cylinder (Corning Glass, Corning, NY) (45.7 cm height, 22.2 cm inside diameter) filled to a height of 38 cm with water at 27 ± 1°C. Animals were immersed for 11 min in fresh tapwater or water previously soiled by another two rats. Animals (n = 11–16/group) were scored for occurrence of generalized forelimb or hindlimb clonus or tonus convulsions (30) and for immobility, the criterion for which was floating in the water, making only those movements necessary to keep the nose above water (10). Behavior during the first minute was considered an adaptation period and immobility times were not recorded. Animals were tested after receiving the last of three injections of imipramine hydrochloride ((Sigma, St. Louis, MO) (0, 7.5, 15, or 30 mg/kg at 24, 5, and 1 h before testing). Animals were given three injections rather than a single injection prior to testing because imipramine's experimental effects are more consistent and pronounced after repeated administrations, as are its therapeutic actions (10).

Surgery

Since the forced-swim test and imipramine both cause increases in serum adrenal hormone levels (2,28), the possibility that the combination of imipramine and stress produce convulsions due to excessive adrenal release was also examined by including adrenalectomized and sham-operated animals in our test paradigm. For adrenalectomy, animals were anesthetized with sodium pentobarbital (50 mg/kg). Bilateral adrenalectomy (ADX) was performed via bilateral lumbar incisions through which adrenals were removed. For sham-treated animals, adrenals were located but not manipulated. Following surgery, ADX animals were placed on 0.9% saline instead of tapwater to prevent dehydration (16). Two weeks after surgery, animals were injected with imipramine and tested in the forced-swim test. Animals were sacrificed after testing and examined for remaining adrenal tissue. Animals with remaining tissue were not included in the data analysis. Group differences in number of animals convulsing were examined by χ^2 analysis. Group differences in immobility time were examined by analysis of variance (ANOVA).

RESULTS

The data are shown in Table 1. There was no significant effect of adrenalectomy on convulsions and therefore treatment groups were combined for statistical purposes. None of the animals tested in fresh water convulsed. In contrast to this observation, the combination of imipramine treatment and testing in soiled water was significant (χ^2 = 41.9, df = 3, $p < 0.001$) with 8 (3 adrenalectomized) of the 11 animals given the combination of the high dose of imipramine and testing in soiled water having a clonic convulsion.

Excluding animals that convulsed, animals tested in soiled water exhibited almost no immobility, $F(1,98) = 149$, $p < 0.0001$). Neither adrenalectomy nor imipramine had a significant effect on this outcome. We also did not observe a dose-response effect. This may have been due to our increments in dosage levels or that the threshold for this effect begins at the 30-mg/kg dose level.

DISCUSSION

The decreased immobility time of animals tested in soiled water, which presumably contains an alarm chemosignal, corroborates our previous observation (1,3–5). This effect was not influenced by adrenalectomy or imipramine.

This is not the first report in which an alarm chemosignal has been found to have the potential for inducing convulsions. Cocke and Thiessen (13) reported that two gerbils exposed to odors from the blood of another stressed gerbil immediately developed tonic-clonic seizures. However, this anecdote, to our knowledge, has never been formally evaluated. Neither imipramine itself nor testing animals in fresh water was sufficient for convulsions to occur; both conditions were necessary.

This observation may shed some light on a puzzling clinical side effect of imipramine. Tremors and seizures are among the common adverse reactions of tricyclic antidepressants such as imipramine and can occur in individuals with no previous seizure history or other known neurological dysfunction [e.g., (26)] and in children receiving tricyclic antidepressants for treatment of enuresis or hyperactivity [e.g., (34)]. The pathophysiology for these convulsions is still unknown. While many environmental factors have been found to affect the likelihood of seizure activity (15,18,23) emotional stress has not received much attention as a precipitating factor although there are several anecdotal and survey reports of such an association [e.g., (17,19,29,32,36)]. The present study suggests that, while imipramine does not typically produce convulsions, if patients are experiencing considerable emotional stress the combination of imipramine and stress may result in a convulsion.

The occurrence of convulsions in adrenalectomized animals indicates that corticosterone is not involved although both imipramine and stress cause increases in plasma cortico-

TABLE 1

EFFECTS OF WATER CONDITION AND IMIPRAMINE ON CONVULSIONS AND MEAN IMMOBILITY TIME (± SE)

Dose (mg/kg)	Water Condition			
	Fresh		Soiled	
	No. Convulsing/(n)	Immobility Time (s)	No. Convulsing/(n)	Immobility Time (s)
0	0/16	251 ± 35	0/16	12 ± 10
7.5	0/16	304 ± 24	0/16	6 ± 2
15.0	0/18	265 ± 35	0/18	32 ± 12
30.0	0/11	380 ± 20	8/11	0 ± 0

Treatment groups (control, sham, adrenalectomy) were not statistically different and were combined.

sterone levels (3,28). A possible hypothesis we are currently exploring is that the convulsive effect of combined stress and imipramine is due to their common actions at central noradrenergic and serotonergic receptors (6,21).

In more general terms, the observation of convulsions in animals experiencing a combination of stress and imipramine suggests both a new animal model for studying convulsions and an empirical basis for some convulsive disorders.

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