Pharmacology Biochemistry and Behavior, Vol. 45, pp. 561-563, 1993

0091-3057/93 $6.00 + .00

Strain Differences in Convulsions Induced by Alarm Substance

ERNEST L. ABEL

Department of Obstetrics and Gynecology and
Fetal Alcohol Research Center, Wayne State University, 275 E. Hancock, Detroit, MI 48201

Received 15 January 1993

ABEL, E. L. *Strain differences in convulsions induced by alarm substance.* PHARMACOL BIOCHEM BEHAV **45**(3) 561-563, 1993.—Rats exhibit considerable immobility in the forced swim test when tested in fresh water but almost no immobility when tested in soiled water, that is, water previously swum in by another rat. In this study, we examined the response of different strains of rats to soiled water after treatment with imipramine, which precipitates convulsions in rats tested in soiled water. Male Long-Evans, Sprague-Dawley, Wistar, or Fischer rats were injected with imipramine (20 or 30 mg/kg, IP) and subsequently tested in water soiled by either a Sprague-Dawley or Wistar rat. Significantly more animals convulsed when the soiled water was produced by a Sprague-Dawley rat than a Wistar. More rats also convulsed after receiving the 30-mg/kg dose. Different strains did not react differently to soiled water. This study corroborates and extends previous findings that alarm substances can induce convulsions in imipramine-treated rats and indicates that some strains produce a more potent alarm substance than others.

Alarm substance Convulsions Imipramine Rats

IN the forced swim test (17), rats are immersed in a cylinder of fresh water from which they cannot escape. The following day, they are reimmersed and, typically, they are more immobile during the retest session than during the same test period on the previous day. We adapted this test in two ways. One of these adaptations involves testing animals only during the initial immersion; the second involves testing animals in either fresh water or soiled water, that is, water previously swum in by another rat, which may contain urine, feces, skin oils, saliva, sweat from foot pads, or other excreta. When tested in fresh water, rats initially paddle vigorously for a few minutes but eventually become immobile and adopt a characteristic floating response. In soiled water, rats also paddle vigorously when initially placed in the apparatus, and although these movements decrease in intensity rats do not maintain a floating response (1-6). Because stressors such as prior foot-shock or loud noise also disrupt immobility (5,8), we have interpreted lack of immobility as a stress response [cf. (11,15)] and have hypothesized that soiled water contains an alarm substance that acts like foot-shock or loud noise, to disrupt the immobility response typically seen when animals are not prestressed before testing (1-6).

We have not isolated the hypothetical alarm substance in soiled water as yet but described some of its characteristics. For example, we found that the proposed alarm substance can be detected as long as 8 days after it has been released (1), its effects are concentration related (1), it is not contained in feces or urine (5), it has low volatility (3), it is not readily depleted by repeated testing (1), and it is not dependent upon the adrenal glands for production (6). In a recent study, we also found some strains are affected by the putative alarm substance more, for example, Sprague-Dawley and Long-Evans, than others, for example, Wistar and Fischer, in term of their immobility response, and some strains, for example, Sprague-Dawley rats, produce a much more potent alarm substance than others, for example, Wistar rats, in terms of its suppressant effects on immobility.

In a related study, we also found that while imipramine reduces immobility in fresh water, as reported by Porsolt et al. (17), if rats are tested in soiled water imipramine can also induce convulsions. We conducted the present study to determine if, like the immobility response, there are strain differences in terms of the production or reaction to soiled water on the part of imipramine-treated rats. Because imipramine, a drug widely used in treatment of depression, also causes convulsions in patients with no previous seizure history (16,19), the present study, exploring strain differences in response to imipramine's convulsive effects, was conducted to clarify a possible genetic component to this phenomenon and to extend the heuristic value of this model in exploring the pathophysiology for idiopathic convulsions associated with imipramine.

For these studies, we focused on Sprague-Dawley and Wistar rats as "producers" of the putative alarm substance, that is, the sources for the alarm substance, because we previously found that the effect of their putative alarm substance on immobility is significantly different, with that produced by Sprague-Dawley rats having the greatest suppressant effect on

the immobility response (4), and we tested four strains of rats as "receivers" to determine their reaction to the alarm substance produced by these two strains.

METHOD

Four strains of male rats were studied for their convulsive reaction to alarm substance: Long-Evans, Wistar, Sprague-Dawley (Charles River, Portage, MI) and Fischer 344 (Charles River, Raleigh, NC). Animals were 70–80 days of age when tested. Animals 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 (light on at 7:00 a.m.). Food and water were available ad lib.

Animals were tested after receiving the last of three injections of imipramine HCl (Sigma Chemical Co., St. Louis, MO) (20 or 30 mg/kg, IP, 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 (11). Animals were only tested in soiled water because they do not convulse in fresh water (4).

For testing, animals were immersed in a Plexiglas cylinder (Corning Glass Works, Corning, NY) (45.7 cm height, 22.2 cm inside diameter) filled to a height of 38 cm with water at 32 ± 1°C, which had previously been soiled, that is, swum in, by a Sprague-Dawley or Wistar male of the same age for 15 min immediately prior to testing. Test animals (7–10/group) were scored for occurrence of generalized forelimb or hindlimb clonic or tonic convulsions (18).

The data for convulsions were analyzed by χ^2 analysis in terms of producers (the two strains soiling the water), receivers, (the four strains being tested), and dosage. The latencies for convulsions were analyzed by analysis of variance (ANOVA) with these three factors as independent factors.

RESULTS

One Wistar rat in both the 20- and 30-mg/kg groups convulsed prior to testing and were not included in the analysis.

The data are presented in Table 1. Significantly more animals convulsed when tested in water soiled by a Sprague-Dawley rat (24 of 57) than by a Wistar rat (8 of 57), $\chi^2(1) = 11.1$, $p < 0.0009$. Strain differences in response to alarm substance were not significant. Significantly more animals convulsed after receiving the 30-mg/kg dose than the 20-mg/kg dose, $\chi^2(1) = 7.9$, $p < 0.005$. Because there was a significant dose effect, we also examined producer and receiver effects at each of these doses. Neither producer nor receiver effects were significant when animals were primed with the 20-mg/kg dose. When primed with the 30-mg/kg dose, significantly more animals convulsed if the water was soiled by Sprague-Dawley rats than by Wistar rats, $\chi^2(1) = 8.3$, $p < 0.004$.

ANOVA was also conducted on the latencies to convulse. For this analysis, nonconvulsing animals were given latencies of 700 s. Mean (±SE) time to convulse for animals tested in water soiled by Sprague-Dawley rats was 561 ± 30 s compared to 635 ± 22 s for Wistar rats, $F(1, 98) = 4.96$, $p < 0.01$. Group differences in receiver latencies to convulse were not significant. Animals treated with the 30-mg/kg dose convulsed much sooner (551 ± 33 s) than those treated with 20 mg/kg (636 ± 21 s), $F(1, 98) = 6.59$, $p < 0.01$. None of the interactions was significant.

DISCUSSION

Alarm substances are chemical signals produced by a wide variety of species (9,10,12,13). Depending upon test conditions, these alarms substances elicit freezing, dispersal, or aggregation in conspecifics (13,20). Alarm substances can also induce physiological changes in conspecifics. For example, Cocke and Thiessen (14) showed that alarm substances can affect immune function in conspecifics. The present study and our previous report (4,6) show that water previously swum in by a rat appears to contain an alarm substance because it elicits a stress-like behavioral response (decreased immobility) and physiological response (convulsions after priming with imipramine) in conspecifics. Although we suspect that this alarm substance is a pheromone, this is still speculative because we have not been able to isolate it. Thus far, we found that filtering soiled water does not affect its effects on immobility but boiling it does eliminate that effect. We also eliminated the adrenal glands as the source for the putative alarm

TABLE 1

EFFECTS OF STRAIN (SPRAGUE-DAWLEY OR WISTAR) PRODUCING ALARM SUBSTANCE (PRODUCERS) ON IMIPRAMINE-PRIMED CONVULSIONS IN FOUR STRAINS OF RATS TESTED IN SOILED WATER (RECEIVERS)

	Producer Strain				
	Sprague-Dawley No. Convulsing/ No. Tested		Wistar No. Convulsing/ No. Tested		Total No. Convulsing/ No. Tested
Receivers	20 mg/kg	30 mg/kg	20 mg/kg	30 mg/kg	
Long-Evans	5/9	5/6	0/7	3/7	13/29
Fisher	1/8	1/6	1/6	1/6	4/26
Wistar	2/9	5/5	0/8	1/7	8/29
Sprague-Dawley	1/8	4/6	1/8	1/8	7/30
Totals for Dose	9/34	15/23*	2/29	6/28*	
Totals for Strain	24/57†		8/57†		

*$\chi^2 = 8.20$, $p < 0.004$.
†$\chi^2 = 11.1$, $p < 0.002$.

substance because adrenalectomy does not alter production or reaction to it (6).

This study extends our previous report by showing that some strains, for example, Sprague-Dawley rats, produce a more potent or greater amount of this substance than others. Using immobility as a dependent variable instead of convulsions, we found a similar significant difference in the reaction to the putative alarm substance produced by these two strains (4). The fact that some strains produce either a more potent or greater amount of this proposed alarm substance indicates its production is not a fundamental characteristic in rats. This, in turn, suggests a possible opportunity to investigate other strain differences in the behavioral and physiological correlates of alarm substance.

The induction of convulsions in imipramine-primed rats represents a new model for studying convulsions in animals. Although studies using this model are still preliminary, they may be of significance for understanding the pathophysiology of convulsions that occur in individuals receiving this medication that have no previous seizure history (16,19).

ACKNOWLEDGEMENTS

The author thanks D. Waselewsky, C. Zajac-Benitez, S. Kubinek, and B. Abel for technical assistance.

REFERENCES

1. Abel, E. L. Gradient of alarm substance in the forced swimming test. Physiol. Behav. 49:321–323; 1991.
2. Abel, E. L. Behavior and corticosteroid response of Maudsley reactive and nonreactive rats in the open field and forced swimming test. Physiol. Behav. 50:151–153; 1991.
3. Abel, E. L. Alarm substance emitted by rats in the forced swim test is a low volatile pheromone. Physiol. Behav. 50:723–727; 1991.
4. Abel, E. L. Response to alarm substance in different rat strains. Physiol. Behav. 51:345; 1992.
5. Abel, E. L.; Bilitzke, P. J. A possible alarm substance in the forced swimming test. Physiol. Behav. 48:233–239; 1990.
6. Abel, E. L.; Bilitzke, P. J. Adrenal activity does not mediate alarm pheromone reaction in the forced swim test. Psychoneuroendocrinology 17:255–259; 1992.
7. Abel, E. L.; Bilitzke, P. J.; Cotton, D. B. Alarm substance induces convulsion in imipramine-treated rats. Pharmacol. Biochem. Behav. 41:599–601; 1992.
8. Amario, A.; Gill, M.; Marti, J.; Pol, O.; Balasch, J. Influence of various acute stressors on the activity of adult male rats in a holeboard and in the forced swim test. Pharmacol. Biochem. Behav. 39:373–377; 1991.
9. Baldwin, I. T.; Schultz, J. C. Rapid changes in tree leaf chemistry induced by damage: Evidence for communication between plants. Science 221:277–279; 1983.
10. Bardach, J. E.; Todd, J. H. Chemical communication in fish. Adv. Chemorecept. 1:205–240; 1970.
11. Borsini, F.; Meli, A. Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology (Berl.) 94:147–160; 1988.
12. Burghardt, G. M. Chemical perception in reptiles. Adv. Chemorecept. 1:241–308; 1970.
13. Cocke, R.; Thiessen, D. D. Chemocommunication among prey and predator species. Anim. Learn. Behav. 14:90–92; 1986.
14. Cocke, R.; Thiessen, D. D. Alarm odors suppress the immune system. In: Macdonald, D. W.; Muller-Schwartz, D.; Natynczuk, S. E., eds. Chemical signals in vertebrates. vol. 5. New York: Oxford University Press; 1990:125–131.
15. Hawkins, J.; Hicks, R. A.; Phillips, N.; Moore, J. D. Swimming rats and human depression. Nature 274:512; 1978.
16. Peck, A. W.; Stern, W. C.; Watkinson, C. Incidence of seizures during treatment with tricyclic antidepressant drugs and bupropion. J. Clin. Psychiatry 44:197–201; 1983.
17. Porsolt, R. D.; LePichon, M.; Jalfre, M. Depression: A new animal model sensitive to antidepressant treatments. Nature 266: 730–732; 1977.
18. Sobrian, S. K.; Nandedkar, A. K. N. Prenatal antiepileptic drug exposure alters seizure susceptibility in rats. Pharmacol. Biochem. Behav. 24:1383–1391; 1986.
19. Tribble, J.; Weinhouse, E.; Garland, J.; Wendelberger, K. Treatment of severe imipramine poisoning complicated by a negative history of drug ingestion. Pediatr. Emerg. Care 5:234–237; 1989.
20. Wilson, E. O. The sociogenesis of insect colonies. Science 228: 1489–1495; 1985.