Pergamon

Pharmacology Biochemistry and Behavior, Vol. 51, No. 4, pp. 849–853, 1995

0091-3057/95 $9.50 + .00

0091-3057(95)00057-7

Behavioral Characteristics of SART-Stressed Mice in the Forced Swim Test and Drug Action

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Received 22 July 1993

HATA, T., E. ITOH AND H. NISHIKAWA. *Behavioral characteristics of SART-stressed mice in the forced swim test and drug action.* PHARMACOL BIOCHEM BEHAV 51(4) 849–853, 1995.—SART-stressed (repeated cold-stressed) mice exhibited shortened immobility time in forced swimming tests, and a time-dependent increase in the duration of immobility time of stressed mice was less compared to unstressed mice. These changes were blocked by diazepam and alprazolam without influence on the immobility time of unstressed mice. The shortening of immobility time caused by SART stress was inhibited by repeated pretreatment with imipramine and mianserin, but not by a single dose. In contrast, neither single nor repeated administrations of lithium carbonate had effect on the immobility time of SART-stressed mice. The SART stress technique may be a potential model to investigate the relationship between stress and depression with complex symptoms like excessive emotion- and anxiety-related depression.

Stress SART stress Forced swimming test Anxiolytics Antidepressants Depression Adaptability

DEPRESSION in humans is increasing, shows various symptoms, and has received considerable attention. Many theories have been advanced to explain depression, but the underlying mechanism is unclear.

The monoamine theory states that depression may be due to reduced levels of brain monoamines or neuronal activity, based on primary pharmacological effects of antidepressants (3,4,32). Studies by chronic administrations of antidepressants in animals show subsensitivity in postsynaptic β-adrenoceptors (2) and decrease in dopamine D_2-receptors (5). The monoamine theory does not account for this.

Stressful events are frequently associated with depression (1), and many similarities are found between behavioral patterns due to chronic stress and symptoms due to depression (6,7,17,36). Using a chronic stress model, the relation between stress and depression has been studied, but so far, little clarification has been achieved.

We investigated the behavioral characteristics of animals exposed to SART (specific alternation of rhythm in temperature) stress (repeated cold stress) (24), a model of autonomic imbalance due to sudden changes in environmental temperature (23). SART-stressed animals showed decreased acetylcholine (20) and serotonin (8) and increased norepinephrine and dopamine (12) in various brain areas in addition to various physiological abnormalities (11,13,14,16,21,22). Abnormal behavior in relation to these changes in neurotransmitters was also noted (9,15).

The present study was carried out to examine whether SART stress produces attributes of an animal model that are prevented by treatment with antidepressants. We studied behavioral characteristics of SART-stressed mice in forced swimming tests and evaluated the effects of anxiolytics and antidepressants.

METHOD

Animals and Stress Procedure

Male ddY mice (Japan SLC. Inc., Hamamatsu, Japan), weighing 22–25 g at the start of the experiments, were used. They were usually housed in a temperature- and light-controlled room (24 ± 1°C, with a 12 L : 12 D cycle, starting at 0700 h) with free access to food (MF, Oriental Yeast, Tokyo, Japan) and water at all times. For SART-stress loading, 8–10 mice per group were alternately transferred to two cages (21.6 × 31.6 × 13.0 cm) in rooms maintained at 24 and 4°C, respectively, at 1-h intervals from 0900 to 1600 h, and housed in a cage kept in the cold room between 1600 and 0900 h overnight, according to the procedures reported previously (10). These procedures were continued for more than 5 days. The stressed mice were subjected to the experiments 1 or more

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hours after getting out of the cold room at 1100 h on the final morning. During the period of stress loading, control mice received no treatment except for handling during daily cage cleaning.

Forced Swimming Test

The swimming tests were conducted according to Porsolt et al. (29). Individual mice were forced to swim in glass cylinders (8 cm in diameter and 20 cm in height) containing fresh water at 21-23°C to a height at 8 cm. Being their first time in the cylinders, the mice vigorously swim around. A few minutes later, their activity began to subside; they eventually became immobile, and floated in the water in an upright position, making only small movements to keep their heads above the water. The time for immobility was measured for 5 min at 30-s intervals, or for 10 min at 1-min intervals. When two mice were subjected to the swim test at the same time, the cylinders were enclosed with opaque panels on three sides of the cylinder to prevent them from seeing each other.

Drugs

Diazepam (Wako Pure Chemical Industries, Osaka, Japan) and alprazolam (a gift from Takeda Pharm. Co., Osaka, Japan), both water-insoluble, were suspended in 0.5% CMC-Na solution and orally administered to mice. Imipramine hydrochloride (Sigma, St. Louis, MO), mianserin hydrochloride (a gift from Organon, Holland), and lithium carbonate (Wako) were dissolved in 0.9% NaCl solution and given IP to mice. Control animals received the vehicle only. These drugs were administered only one time 60 min before the test, or once daily during 6 days of SART stress, six times in all, and the swim test was performed on the day following the final dose according to our previous studies (15,26), in which these administration schedules were convenient. Unstressed mice were also treated with drugs according to the same schedule.

Statistical Analysis

Data were expressed as means with SE. Two-group data were analyzed by the Student's *t*- or Mann-Whitney's *U*-test, and one-way analysis of variance (ANOVA) followed by the Newman-Keuls' test or Duncan's test was used for multigroup data, which were normally distributed according to Bartlett's test. Differences with $p < 0.05$ were considered statistically significant.

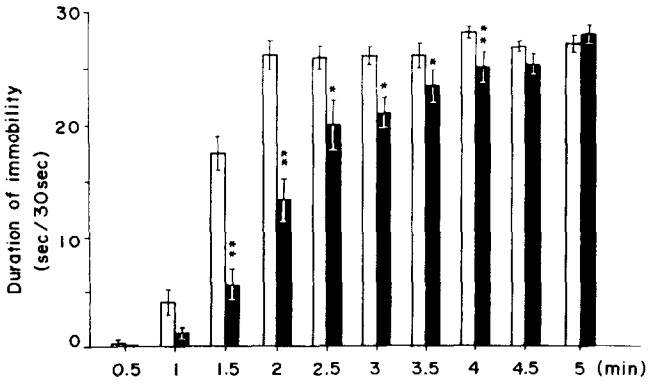

FIG. 1. Immobility time in the forced swimming test in unstressed and SART-stressed mice [1]. Each column represents means ± SE of 20 unstressed (□) or 30 SART-stressed mice (■). Data were nonparametric, and analyzed by Mann-Whitney's *U*-test. *$p < 0.05$ and **$p < 0.01$, compared to the corresponding unstressed group.

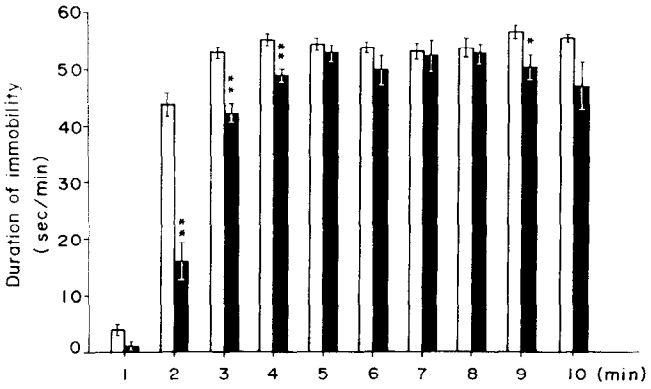

FIG. 2. Immobility time in the forced swimming test in unstressed and SART-stressed mice [2]. Annotations are the same as for Fig. 1.

RESULT

Changes in Immobility Time in SART-Stressed Mice in Forced Swimming Tests

Figures 1 and 2 show the results for every 30-s and 1-min interval in the forced swimming test, respectively. In water, unstressed mice rapidly became immobile after a brief burst of swimming. Two and more min after the start, the mice remained immobile for approximately 80% of the interval and immobility time reached a plateau.

In contrast, stressed mice vigorously swam around, and exhibited significantly ($p < 0.05$ vs. unstressed mice) shortened immobility time during each epoch from 1.5 min to 4 min after the start. The immobility time of stressed mice gradually increased and reached a plateau 5 min later.

As shown in Table 1, stressed mice showed significantly shorter immobility time for 5 min compared with unstressed mice, and also, for 10 min ($p < 0.001$, *t*-test). The ratios between values of stressed group to unstressed group were 0.790 for 5 min and 0.881 for 10 min. Thus, the same data were obtained by test for 5 and 10 min. Due to these results, subsequent measurements were conducted for 5 min.

Figure 3 shows daily changes in immobility time of mice forced to swim for 5 min, once daily for 10 days. The immobility time of unstressed mice gradually increased and reached

TABLE 1

MEAN IMMOBILITY TIME OF MICE IN THE FORCED SWIMMING TEST

	Duration of Immobility (s)		
	Unstress	SART Stress	Ratio
5 min	208.4 ± 4.5	164.7 ± 5.7*	0.790
10 min	481.6 ± 7.0	424.6 ± 9.2*	0.881

Data show the means ± SE of 20 unstressed and 30 SART-stressed mice.

*$p < 0.001$, compared to the unstressed group (*t*-test).

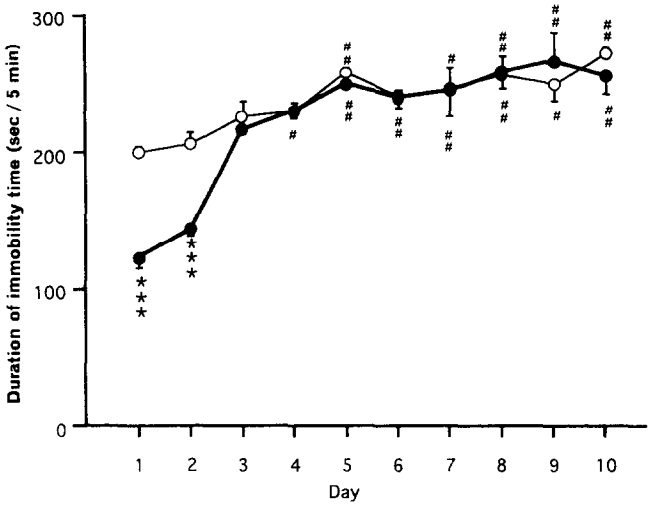

FIG. 3. Time-related changes in immobility time in the forced swimming test in unstressed and SART-stressed mice. Each point represents means ± SE of seven unstressed (○) and seven SART-stressed mice (●). In the stressed group, the mice were already loaded with SART stress for 5 days before day 1 for the forced swim test. SART-stress loading was continued to day 10 for the swim test. Data were parametric. ***$p < 0.001$, compared to the corresponding unstressed value (Student's t-test). #$p < 0.05$ and ##$p < 0.01$, from the respective day 1 value (Duncan's test).

a plateau on day 3, and there were no significant differences in the data of day 3 to day 9 from each other (Duncan's test). The time of stressed mice was significantly shorter than that of unstressed mice in day 1 and day 2 ($p < 0.001$, Student's t-test). Thereafter, it rapidly increased and reached a plateau on day 4, and no significant differences could be found among the data for day 4 to day 10 (Duncan's test).

Effects of Drugs on Shortened Immobility Time Due to SART Stress

Figure 4 shows the effects of anxiolytics on immobility time in the forced swim test in mice. Diazepam and alprazolam dose dependently normalized the shortened immobility time of SART-stressed mice by single doses without the influence on unstressed mice. The results obtained with the higher doses of these drugs were significantly different from those of the respective stressed control [ANOVA $F(7, 52) = 3.809$, $p < 0.01$ for diazepam; $F(7, 85) = 7.030$, $p < 0.001$ for alprazolam].

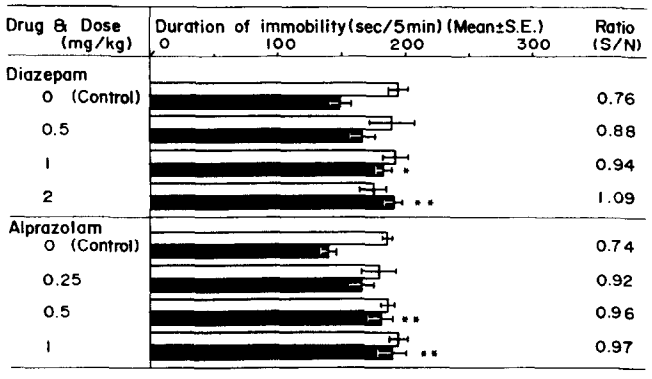

FIG. 4. Effects of anxiolytics on shortened immobility time in SART-stressed mice in the forced swimming test. Each column represents means ± SE of 10–13 mice/control group and 4–13 mice/dosed group. N, □ = unstress; S, ■ = SART stress. The data were parametric by Bartlett's test, and analyzed by the one way ANOVA following by Newman-Keuls' test. *$p < 0.05$ and **$p < 0.01$, compared to the respective control group.

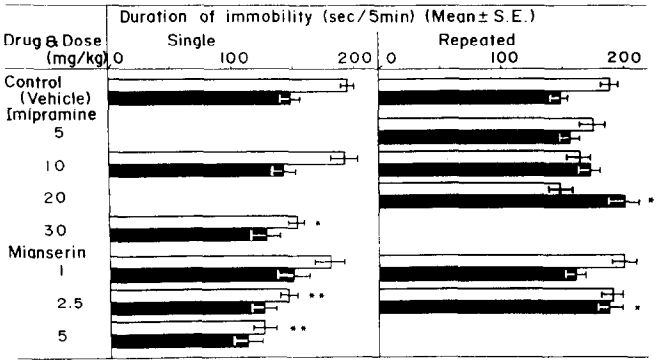

FIG. 5. Effects of antidepressants on shortened immobility time of SART-stressed mice in the forced swimming test. No. of mice: 13–20 mice/control group and 5–12/dosed group. Other annotations are the same as for Fig. 4.

As shown in Fig. 5, single administration of imipramine shortened the immobility time of unstressed mice, but not that of stressed mice [ANOVA $F(5, 51) = 8.887$, $p < 0.001$]. Single doses of mianserin dose dependently shortened the time of unstressed mice and reduced even the shortened immobility time caused by SART stress [ANOVA $F(7, 61) = 9.954$, $p < 0.001$].

On the right side of Fig. 5, daily doses of imipramine [ANOVA $F(7, 83) = 3.245$, $p < 0.01$] and mianserin [ANOVA $F(5, 59) = 7.818$, $p < 0.001$] can be seen to prolong and normalize shortened immobility time in SART-stressed mice. Repeated administrations of imipramine shortened the immobility time of unstressed mice.

The effects of lithium carbonate are listed in Fig. 6. Lithium carbonate, at single or daily doses, had no effect on immobility time in unstressed or SART-stressed mice.

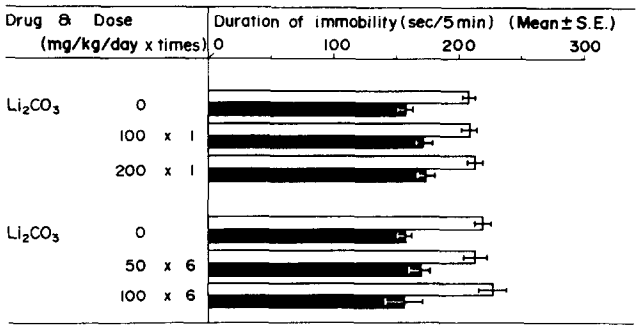

FIG. 6. Influence of lithium carbonate on immobility time of mice in the forced swimming test. Each column represents means ± SE of 6–13 mice/group. □ = unstress, ■ = SART stress. There were no statistical differences between unstressed and stressed data according to the results of one-way ANOVA conducted following Bartlett's test.

DISCUSSION

There are few reports on effects of stress on forced swimming test results. Garcia-Marquez and Armario (6) reported that exposure of rats to uncontrollable shock or a combination of various stressors increased immobility time. In contrast, Platt and Stone (28) found that chronic restraint stress reduced immobility time in forced swimming tests. Thus, the effects of stress on forced-swim tests appear to differ according to the kind of stress. But it appears certain that stress causes abnormal behavior and is closely related to depression.

In the present study, immobility was readily induced in unstressed mice after a short time of immersion in water and 2 min thereafter, the duration of immobility reached a plateau. The duration remained almost constant throughout the experiments after day 3, during which period the unstressed mice were forced to swim once daily. The data on unstressed mice are in agreement with the findings of Porsolt et al. (29,30) and Shimazoe et al. (34). In contrast, SART-stressed mice showed shortened immobility time and daily increase in immobility time for the test period was less compared with that in unstressed mice, not only in the first but subsequent days as well. These results indicate that it is difficult for SART-stressed mice to adapt to a new environment.

Nomura and Naitoh (27), from the standpoint of adaptability to the environment, propose rats kept in an overcrowded environment, as a novel animal model of depression. The rats did not show stress response or physiological symptoms. When exposed to a novel environment, they manifested abnormal behavior including increase in swimming head twitch. These changes were prevented by chronic administrations of antidepressants. Rats without adaptability to chronic restraint stress have also been proposed as an animal model of depression (18,19). Neurochemical similarities have been found in animals in response to antidepressant treatment and stressful stimuli. Antidepressants and stress, when experienced chronically, reduce the density of β-adrenoceptors in various regions of rat brain (33,35). Unadaptability to stress and change in environment would, thus, appear linked to depression. Accordingly, SART-stressed mice may be linked to depression.

The forced swimming test is a well-known screening model for antidepressants developed by Porsolt et al. (31). In this test, immobility was reduced by typical and atypical antidepressants (29–31). Our present results on antidepressants in unstressed mice are consistent with these reports. SART stress reduced the duration of immobility in mice, too. Single doses of antidepressants still more reduced the shortened immobility time due to SART stress. However, repeated administrations of antidepressants dose dependently prolonged and normalized the duration of immobility in SART-stressed mice, in contrast to unstressed mice. These effects of imipramine and mianserin are much the same as those obtained clinically, which is delayed possibly by as much as 2–3 weeks. Single and repeated administrations of lithium carbonate had no effect on the immobility time of stressed mice. It, thus, follows that SART-stressed mice may not be in a state of mania but depression.

Diazepam did not affect the duration of immobility at small doses but prolonged it at large doses (25). In the present study, small doses of diazepam and alprazolam prolonged shortened immobility time due to SART stress but had no influence on that of unstressed mice. Shortened immobility time caused by SART stress would, thus, appear possibly associated with excessive emotion including anxiety. This is not inconsistent with the finding that SART-stressed rats exhibit hyperactivity with increased defecation in open-field test (15).

Based on the present results, SART-stressed mice may be concluded to be in a state of depression accompanied by anxiety and excessive emotion with low adaptability to novel environments.

SART stress derives from sudden changes in environmental temperature that may be experienced in daily life. This is in contrast to other types of experimental stress as restraint stress, electroshock stress, etc. The SART-stressed animal may simulate the depressed state; a simulation that may be useful in studying the relationship between stress and the biology of depression.

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