Depressive State With Anxiety in Repeated Cold-Stressed Mice in Forced Swimming Tests

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ABSTRACT—The effects of various types of stress and drugs were studied to assess mouse performance in forced swimming tests, following characterization of SART (specific alternation of rhythm in environmental temperature) stress. Immobility time in the test decreased in mice subjected to SART, acute cold and restraint stress. No change was noted due to chronic cold stress or repeated fasting. The shortened time did not recover even 24 hr after the end of SART and chronic restraint stress. The time in SART-stressed mice finally recovered at 5–7 days. Shortening of immobility time in SART-stressed mice was inhibited by diazepam and repeated imipramine but not by lithium carbonate. In chronic restraint-stressed mice, this time was inhibited by repeated lithium carbonate but not diazepam or imipramine. SART stress would thus appear related to anxiety and depression and may be useful for detecting new types of antidepressants.

Keywords: Forced swimming test, Stress, SART (specific alternation of rhythm in environmental temperature) stress, Depression, Anxiety

Depression is a very frequent psychiatric disorder (1, 2), and thus appropriate animal models of depression have been sought to determine the mechanisms by which antidepressive drugs exert therapeutic action and characterize the biological basis of depression (3–7). Chronic stress has been found quite useful as a model because life stress is frequently associated with depressive disorders, and the behavioral effects of chronic stress have been shown to be similar to endogenous, mainly bipolar, depression in some respects (8). Individually housed mice have been also found to show depression-like alterations of behavior (9). Rats exposed chronically to unpredictable stress were reported to show a depressive-like state (10, 11).

Porsolt et al. (12, 13) suggested that the characteristic immobility observed in rats and mice during forced swimming reflects a state of despair. This immobility is reduced by various agents therapeutically effective for treating depression. Immobility in forced swimming tests is affected by stress, even though this has been little studied. Exposure of rats to uncontrollable shock or a combination of various stressors increased immobility (5, 6). Chronic restraint stress reduced immobility in forced swimming tests (14), as did also SART (specific alternation of rhythm in environmental temperature) stress (15).

The effects of stress in forced swimming tests thus appear to differ according to the types of stress.

SART stress is the simulation of repeated and sudden changes in environmental temperature that may be encountered by humans in daily life such as early spring or autumn or when leaving an air-conditioned room in summer or winter (16). Animals exposed to SART stress are a model of autonomic imbalance (17), and they show decreased acetylcholine (18) and serotonin levels (19) and increased norepinephrine and dopamine levels (20) in various brain areas in addition to physiological abnormalities and abnormal behavior in the open-field (21) and step-down tests (22, 23). SART-stressed mice were recently found to exhibit shortened immobility time in forced swimming tests and their time-dependent increase in immobility time was less compared to that for non-stressed mice (15).

In this study, the abnormal behavior of SART-stressed mice in the forced swimming test was examined in detail. Attention was also directed to the effects of various types of stress on performance in forced swimming and drugs therapeutically effective for treating depression.

MATERIALS AND METHODS

Animals

Male ddY mice (Japan SLC, Shizuoka), weighing
22–25 g at the start of this study, were used. The animals were housed at room temperature (24±1°C), 12-hr light/dark cycle (lights on 0700 hr, off 1900 hr) with free access to food (MF; Oriental Yeast, Tokyo) and water.

**Stress procedure**

Eight to ten mice per group were alternately transferred to two cages (21.6×31.6×13.0 cm) in rooms maintained at 24°C and 4°C, respectively, at 1-hr intervals from 0900 to 1600 hr, and housed in a cage kept in the cold room between 1600 and 0900 hr overnight, according to the procedures reported previously (24). These procedures were continued for 6 days. The stressed mice were generally used at 1 or more hours after being taken out of the cold room on the final morning.

**Cold stress:** The mice were kept at 4°C for 1 hr, 2 days or 5 days. Experiments were started immediately after stress loading.

**Restraint stress:** Four types of restraint stress were applied: 1) 1-hr restraint stress: Mice were restrained using a metal net for 1-hr from 1300 to 1400 hr in a room maintained at 24°C. 2) 15-hr restraint stress: Mice were restrained using wire cylinders between 1800 and 0900 hr overnight in the room maintained at 24°C. 3) and 4) Chronic restraint stress: Mice received 1-hr or 15-hr restraint stress per day for 5 days repeatedly. Mice subjected to acute stress 1) and 2) were examined immediately after stress loading, and those subjected to two types of the chronic stress were studied at 1 hr after the final stress loading. The control groups were given no food and water according to the same schedule.

**Forced swimming test**

These tests were conducted according to Porsolt et al. (12). Briefly, 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 of 8 cm. The first time, the mice vigorously swam around. A few minutes later, activity began to subside and eventually they ceased to move and floated on the water in an upright position, making only small movement to keep their heads above the water. The time for immobility was measured for 5 min. When two or more mice participated in the swim test at the same time, the cylinders were provided with opaque panels on three sides.

**Drugs**

Diazepam (Wako Pure Chemical Industries, Osaka; water-insoluble) was suspended in 0.5% CMC-Na solution and orally administered to the mice. Imipramine hydrochloride (Sigma, St. Louis, MO, USA) and lithium carbonate (Wako) were dissolved in 0.9% NaCl solution and given i.p. to mice. These drugs were used at doses that had significant influences on stressed mice, but only slight ones on nonstressed mice. The control mice received only the vehicle. The drugs were administered only one time 60 min before the test or administered once daily for 5 days of restraint stress, five times in all; and the swim test was conducted on the day subsequent to the final dose, as in previous studies (22, 23). Nonstressed mice were treated with drugs following the same schedule.

**Statistical analyses**

Data were expressed as means with S.E.M. One-way analysis of variance (ANOVA) followed by the Newman-Keuls' test was used to examine multigroup data, normally distributed as shown by Bartlett's test. Differences of P<0.05 were considered statistically significant.

**RESULTS**

**Effects of stress type on the forced swimming test**

Figure 1 shows the results of forced swimming tests for mice exposed to various types of stress. SART stress

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<th>Duration of Immobility time (sec/5 min)</th>
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Fig. 1. Effects of various types of stress on forced swimming tests in mice. Each column represents the mean immobility time±S.E.M. of 11–19 stressed mice, 6 fasted mice and 54 nonstressed mice. **P<0.01, compared to the nonstressed group (Newman-Keuls' test).
caused significantly shorter immobility time, which, for nonstressed and SART-stressed mice, was 191.0 ± 3.1 and 142.7 ± 9.6 sec, respectively, in agreement with the previous study. The 1-hr and 2-day cold-stressed mice exhibited shortened immobility time. The 5-day cold-stressed mice showed no difference from the nonstressed mice. Mice exposed to acute (1 and 15 hr × 1) and chronic restraint stress (1 and 15 hr/day × 5) showed significantly shorter immobility times compared to nonstressed mice. Repeated fasting had no effect on the forced swimming test results [ANOVA F(10,143) = 16.01, P < 0.01].

Recovery of decreased immobility time following stress application

Figure 2 shows immobility times in mice following stress. The tests were conducted 1 hr after acute stress and 24 hr after chronic stress. SART-stressed mice showed significantly shortened immobility time even 24 hr after the end of stress, which was quite different from the normal level. In contrast to the SART-stressed mice, shortened immobility time in 1-hr and 2-day cold-stressed mice reverted to that of nonstressed mice within 1 hr and 24 hr after stress, respectively. The shortened time in repeatedly 1-hr restraint-stressed mice became normal within 24 hr after stress application but not in repeatedly 15-hr restraint-stressed mice [ANOVA F(6,45) = 8.16, P < 0.01]. Acute (1 and 15 hr) restraint-stressed mice showed normal immobility time 1 hr after stress, in a preliminary test.

Recovery data for decreased immobility time caused by SART stress are given in Fig. 3. The immobility time of SART-stressed mice was significantly shorter than that of nonstressed mice even at 1 and 3 days after stress.

Fig. 2. Recovery from decreased immobility time in forced swimming tests after stress. Each test was conducted one day after cessation of stress except for 1 hr-cold stress in which mice were tested 1 hr after stopping the stress. Each column represents the mean ± S.E.M. of 5–9 mice. **P < 0.01, compared to the nonstressed group (Newman-Keuls’ test).

Fig. 3. Time-related changes in immobility time in forced swimming tests after SART stress. Each point represents the mean ± S.E.M. of 5–9 mice. ○, nonstress; ●, SART stress; □, control levels of non- and SART-stressed mice, respectively. *P < 0.05, **P < 0.01, compared to the nonstressed control and #P < 0.01, compared to the SART-stressed control (Newman-Keuls’ test).
Fig. 4. Effects of diazepam on decrease in immobility time in forced swimming tests on mice exposed to cold or restraint stress. Each column represents the mean±S.E.M. of 6–12 stressed mice and 22 nonstressed control mice. *P<0.05, **P<0.01, compared to the nonstressed control group and #P<0.01, compared to the respective stressed control group (Newman-Keuls’ test).

Thereafter, it rapidly increased and no significant difference from the nonstressed control could be found in the data on day 7.

Effects of drugs on shortened immobility time due to stress

Figure 4 shows the action of anxiolytic, diazepam on shortened immobility time caused by acute (1 hr) cold and restraint stress and chronic restraint (15 hr/day × 5) stress. Diazepam dose-dependently increased immobility time that had been shortened by acute cold and restraint stress. However, shortening by chronic restraint stress was not inhibited by diazepam at 2 mg/kg [ANOVA F(8,102)=13.33, P<0.01]. Effects of diazepam on the time were not tested in nonstressed mice, as it was reported to have no influence on it (15).

The effects of repeated administrations of imipramine and lithium carbonate on shortened immobility time in
chronic 15-hr restraint-stressed mice are indicated in Fig. 5. Daily imipramine slightly shortened the time of non-stressed mice, but lithium carbonate had no influence. Though daily doses of imipramine had no effect on shortened immobility time in chronic restraint-stressed mice, lithium carbonate was found to be effective, and it prolonged and normalized the immobility time [ANOVA F(5,43)=9.77, P<0.01 for imipramine; F(5,62)=16.85, P<0.01 for lithium carbonate].

DISCUSSION

There have been a few studies about the effects of stress in forced swimming tests (5–7, 14, 15). Uncontrollable chronic electric shock (6) and various stressors in combination (5) have been shown to increase immobility time, although chronic restraint stress (14) and SART stress decrease this time (15). The data are thus not consistent, possibly due to different types of stress and/or stress loading schedules. Shortened immobility times in SART-stressed mice was blocked by single doses of diazepam and alprazolam and repeated doses of imipramine and mianserin (15). In this study, the effects of various types of stress were examined in detail on performance in forced swimming, and the changes were found to be unique for SART stress.

No stress in this study prolonged immobility time, and not only SART stress but also acute cold, acute restraint, subchronic cold and chronic restraint stress reduced immobility time. The 1-hr and 2-day cold stress reduced immobility time, but not the 5-day cold stress. Habitation to cold-stress environment may thus possibly develop in 5-day cold-stressed mice. SART stress is a form of chronic stress, but habituation did not occur even after continuous stressing for 2 weeks in SART-stressed animals, and the changes induced by SART stress persisted (18, 20, 25–27). Rats kept in an overcrowded environment were found to show the stress response, and their highly characteristic head-twitching movements in forced swimming were suppressed by repeated desipramine or mianserin but not diazepam or haloperidol. Thus, such stressed rats were proposed as a novel animal model for depression (28). Rats without adaptability to chronic restraint stress have also been proposed as an animal model of depression (4, 7). There is a hypothesis that depression is an unadapted state to stress, from findings that parallel phenomena were found between adaptation to chronic stress and chronic antidepressant treatment (14). From those standpoints of adaptability to the environment and the present findings, SART stress would appear to be linked to depression.

Results in this study and the previous report (15) are shown schematically in Fig. 6. Shortened immobility time caused by acute and chronic cold and acute restraint stress resumed normal values within 24 hr after stress loading. In contrast, SART- and chronic restraint-stressed mice showed a shortened immobility time even at 24 hr after stress loading. This condition in SART-stressed mice persisted for at least three days, and the normal condition was noted at 7 days following stress. The recovery of SART-stressed mice in forced swimming is in agreement with that of other parameters in SART-

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Fig. 6. Schema of drug effects on and recovery from the decreased immobility time of stressed mice in the forced swimming test. The marks † and ↔ indicate, respectively, recovery and no change from decrease in immobility time caused by stress.
stressed animals in previous studies (18, 26, 29, 30). Repeatedly 15-hr restraint-stressed mice, like SART-stressed mice, showed shortened immobility time, and there was no recovery within 24 hr. Shortened immobility time due to SART stress, as well as acute cold and acute restraint stress, was improved by diazepam and by repeated doses of imipramine but not lithium carbonate (15). Some parameters caused by SART stress were already found to be normalized by diazepam and alprazolam (15, 21, 22, 27). The shortened time caused by chronic restraint stress was improved by daily doses of lithium carbonate but not diazepam and repeated imipramine in this study. Imipramine shortened the immobility time of nonstressed mice in the forced swimming test, but lithium carbonate did not, as in the previous report (15). Different types of stress and/or schedule would thus appear to determine immobility time in forced swimming, and abnormal behavior due to stress, at least SART stress, may in some respects be related to a depressive state and/or anxiety. It is interesting that chronic restraint-stressed mice recovered when treated by repeated lithium carbonate in contrast to SART-stressed mice, and this will be tested in the future.

Depression shows various symptoms and is frequently accompanied by anxiety (31, 32). There is a large overlap for anxiety and depression. The international classification of diseases and related health problems (10th version) shows a new diagnostic category, that is, depressive disorder mixed with anxiety, which is not included in both categories of anxiety and depression. Patients with anxiety and depression show severe social impairment, and prognosis is generally poor in spite of early recognition and treatment. The underlying mechanism for this is unclear.

The present results demonstrate abnormal behavior due to SART stress to apparently be related to anxiety and/or depression, in contrast to chronic restraint stress. A depressive state with anxiety may be characteristic of SART stress, which possibly may be useful for detecting new types of antidepressants, but additional study is needed to confirm this.

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