Impairment of Passive Avoidance Performance in SART-Stressed Mice and the Action of Drugs

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Abstract—In order to investigate the behavioral characteristics of the SART-stressed (repeated cold-stressed) animal, a model of dysautonomia, step-down passive avoidance performance was examined in SART-stressed mice. SART-stressed mice exhibited a shortened test trial latency and a decreased incidence of maximum latency of 300 sec, but no change in the training latency. These alterations were blocked by single administration of chlorpromazine or carpipramine prior to the training trial. Repeated, but not single treatments with neurotropin and hopantenate improved the impaired performance due to SART stress. On the other hand, alprazolam and diazepam were ineffective by either mode of administration. Thus, SART-stressed mice appear to have impairment in the process of acquisition of a passive avoidance task.

A number of studies have indicated that stress can modify the escape and/or avoidance behavior of experimental animals (1-4). In an experiment using a 1-trial passive avoidance task, for example, mice exposed to immobilization stress immediately after the training trial exhibited a shortened test trial latency 24 hr later (1). Weiss and Glazer (2) and Weiss et al. (3) reported that acute exposure of rats to cold-water swimming or inescapable electric footshock impaired the acquisition of escape or avoidance performance in a shuttle-box active avoidance paradigm, whereas repeated exposure for 14 days did not. Similar findings were obtained by Nomura et al. (4) when rats were subjected to stress consisting of immobilization and electric shock.

Generally, escape and avoidance behavior is manifested when organisms are motivated to protect themselves from critical situations or noxious stimuli (5). Therefore, it is of interest that such behavior was affected by acute but not chronic stress.

As part of the pathophysiological examination of animals loaded with SART (specific alternation of rhythm in temperature) stress (6), a chronic type of stress, the authors have been investigating the behavioral characteristics of SART-stressed animals, accepted as an animal model of dysautonomia (7). SART-stressed animals have been reported to exhibit a decreased acetylcholine content (8) and increased contents of norepinephrine and dopamine (9) in various brain areas. Since these neurotransmitters are known to be associated with the manifestation of various forms of behavior, and such animals also exhibit abnormal electrocorticograms (10), the abnormal behavior of SART-stressed animals can be predicted with reasonable accuracy. In an attempt to investigate the characteristics of avoidance behavior in such animals, the present study examined passive avoidance responses in SART-stressed mice and, furthermore, evaluated the actions of drugs upon them.

Materials and Methods

1. Animals and stress procedure: The animals used were male ddY mice (Shizuoka Laboratory Animal Center) weighing 20-30
They were housed in a temperature- and light-controlled room (24±1 °C, with a 12-hr light-dark cycle starting at 07:00) with free access to food (MF, Oriental Yeast) and water at all times.

For producing SART-stressed animals (11), mice were alternately exposed to room temperatures of 24°C and 4°C at 1-hr intervals from 09:00 to 16:00 and then kept at 4°C from 16:00 to 09:00 the following morning. This procedure was repeated for 5 consecutive days, and then the stressed mice were subjected to experiments on the 6th day.

2. Passive avoidance task: The passive avoidance response was assessed according to the step-down method (12). The test apparatus consisted of a transparent plastic box (16 x 14 x 16 cm), an electronic stimulator (SEN-3201, Nihon Kohden) and an isolator (SS-102J, Nihon Kohden). The experimental box had an electrifiable grid floor consisting of 19 stainless steel bars having a diameter of 2 mm placed 8 mm apart, which was connected to the stimulator via the isolator, and a wooden platform (5 x 5 x 3.5 cm) was placed in one corner.

In the training trial, each mouse was placed on the platform with its head toward the corner, and as soon as all 4 limbs had touched the grid floor, an electric shock current (5 mA, 50 Hz) was delivered to the floor grid for 5 sec. The mouse was then returned to its home cage. Step-down latency was defined as the time from the moment the mouse was placed on the platform until it stepped down. The training session was given a cut-off limit of 30 sec, although most of the mice in this study stepped down within this time limit.

The test trial was performed in the same manner 24 hr following the training trial. In this case, the upper limit of latency was 300 sec, and mice that failed to respond within the time limit were assigned a maximum step-down latency of 300 sec. In addition, the percentage of mice showing a step-down latency of 300 sec was calculated. All experiments were carried out between 12:00 and 17:00 in a soundproof chamber.

3. Drug administration: The drugs used were alprazolam (Takeda), diazepam (Wako), chlorpromazine hydrochloride (Shionogi), carpiptramine dihydrochloride monohydrate (Yoshitomi), Neurotropin® (an extract from the inflamed skin of rabbits treated with vaccinia virus, Nippon Zoki, 10 mg/ml) and calcium hopantenate (Tanabe, Hopate®).

Alprazolam and diazepam were suspended in 0.5% CMC-Na solution and administered orally to mice. Chlorpromazine, carpiptramine and hopantenate were dissolved in 0.9% NaCl solution. These drugs and neurotropin were injected i.p. into mice. Control animals were given the vehicle only.

For examining the acute effects of these drugs, mice received a single dose of each drug 1 hr before the training trial. In the chronic studies, the drugs were administered between 11:00 and 12:00 once daily during 5 days of SART stress, 5 times in all, and the training trial was performed on the day following the final dose. Unstressed mice were also treated with the drugs according to the same schedule.

4. Statistical analysis: The step-down latencies obtained were expressed as means±S.E., and statistical comparisons between groups were performed by the two-tailed Mann-Whitney's U-test. The incidence of a maximum latency of 300 sec in the test trial was statistically analyzed by the two-tailed Fisher's exact probability test.

Results

1. Passive avoidance response in SART-stressed mice

Figure 1 illustrates the results of the passive avoidance test in unstressed and SART-stressed mice. No difference was evident in the training trial between the step-down latencies of non-stressed and SART-stressed mice. Some mice in both groups were given no electric shock in order to make a subsequent comparison.

In the test trial, non-shocked mice showed step-down latencies similar to those in the training trial in both non-stressed and SART-stressed mice. Some mice in both groups were given no electric shock in order to make a subsequent comparison.

In the test trial, non-shocked mice showed step-down latencies similar to those in the training trial in both non-stressed and stressed groups. In the shocked groups, on the other hand, unstressed mice showed a test latency of 233.3 sec, which was much longer than the training latency. Also, the test trial latency of stressed mice was 136.7 sec, which was significantly (P<0.01) longer than their training latency, but significantly shorter than the test latency of unstressed mice. The per-
percentage of mice showing a maximum test latency of 300 sec was 5% in the stressed group, which was significantly smaller than the figure of 50% in the unstressed group.

2. Effects of drugs on the impaired passive avoidance response in SART-stressed mice

As a similar tendency was shown, as mentioned above, in both changes in the test latency and the percentage of mice showing a maximum latency of 300 sec, only the change in the test latency was used as a parameter to evaluate the drug effect in the following studies.

a) Acute effect: Table 1 indicates the training latency following single doses of test drugs. Most of the drugs examined did not
affect the training latency at any dose employed, except that carpipramine at 10 mg/kg significantly prolonged the latency in SART-stressed mice.

The results obtained in the test trial are presented in Fig. 2.

As seen in Fig. 2, the antianxiety drugs, alprazolam and diazepam, shortened the test latency of unstressed mice at the respective highest doses. Similarly, in SART-stressed mice, the two drugs shortened or tended to shorten the test latency.

The antipsychotic drugs chlorpromazine and carpipramine inhibited the shortening of test latency caused by SART stress in a dose-dependent manner, without any influence on unstressed mice.

The sedative analgesic neurotropin at the doses evaluated in this study failed to affect passive avoidance performance in either unstressed or stressed mice.

Hopantenate, which has been used clinically for treating patients with amnesia, caused a shortened test latency in non-stressed mice at a dose of 1,000 mg/kg, but did not affect the impaired avoidance behavior in SART-stressed mice.

b) Chronic effect: Since alprazolam, diazepam, neurotropin and hopantenate in single doses had no effect on the impaired passive avoidance performance due to SART stress, the effect of repeated treatments with these drugs was evaluated in the subsequent experiments.
The training latency in both unstressed and SART-stressed mice was unaffected by daily treatments with any of the drugs examined (data not shown).

Figure 3 shows the results of the test trial. Daily administrations of alprazolam and diazepam shortened the latency in non-stressed mice but did not do so in stressed mice.

Both neurotropin and hopantenate dose-dependently normalized the shortened test latency caused by SART stress without any notable effect on unstressed mice.

**Discussion**

The present experiments showed that SART stress can cause alterations in step-down passive avoidance performance in mice including a shortened test-trial latency and a decreased percentage of mice showing a maximum latency of 300 sec. It has been suggested that altered sensitivity to electric shock may affect avoidance behavior (13). However, reduced sensitivity to shock is unlikely to have been responsible for the impaired avoidance performance seen in this study, because SART stress has been reported to produce hyperalgesia in mice (14), differing from other common stresses that induce analgesia.

In passive avoidance paradigms, alterations in the response latency have been thought to reflect the degree of memory and therefore have been utilized for the testing of anti-amnesic agents. However, since passive avoidance performance results from the memory of fear and/or anxiety, the emotionality of animals can presumably affect the avoidance behavior. In the present study, the test latency in SART-stressed mice receiving a shock in the training trial was much longer than that in non-shocked mice, suggesting that SART-stressed animals retained, at least, the memory of the shock experience. It should be mentioned here that SART-stressed animals exhibited hypersensitivity to external stimuli in the GSR (galvanic skin response) test (6), resting-arousal electrocorticograms with low-voltage fast waves (10), and excessive behavioral activity accompanied by increased defecation in the open-field test. Taken together, it is considered that the impaired avoidance performance due to SART stress may be associated with excessive emotionality and excessive behavioral activity. In other words, SART-stressed animals are in a state of excessive emotional and behavioral activity, resulting in a situation that makes it difficult for them to normally perform the passive avoidance task.

Single administration of chlorpromazine or carpiramine prior to the training trial improved the impaired performance in the passive avoidance paradigm due to SART stress. It is generally accepted that these drugs exhibit their actions by blocking norepinephrine and dopamine receptors (15). SART-stressed animals have been reported to exhibit increased levels of norepinephrine and dopamine in various brain regions (9). Then, it seems likely that the impaired passive avoidance performance due to SART stress was produced through alterations in the function of the catecholaminergic system. In addition, considering that these drugs were administered prior to the training trial, thereby producing an effect, SART-stressed mice would appear to have an abnormality in the process of acquisition of a passive avoidance task.

Neurotropin and hopantenate, when administered repeatedly, prevented the SART stress-induced impairment in passive avoidance performance. Hopantenate has been shown to exert its improvable effect on scopolamine-induced impairment in the passive avoidance task, and it is suggested that the effect may be due to activating the cholinergic system via the GABAergic system (16, 17). In addition, it has been suggested that hopantenate has no effect on the dopaminergic system (18). On the one hand, since SART-stressed animals have been reported to show the reduced acetylcholine content in various brain areas (8), it is also possible that the passive avoidance impairment due to SART stress may be associated with altered functions of the cholinergic system, besides the catecholaminergic system. Numerous previous studies (19–23) have shown that neurotropin is effective in moderating most of the abnormal SART stress-induced symptoms including alterations in the contents of brain neurotransmitters (19, 22). Therefore, neurotropin is thought to alleviate SART stress, thereby improving the impaired avoid-
ance performance. On the other hand, the recent finding (24) that the analgesic effect of neurotropin is related to the GABAergic system leads to the speculation that neurotropin, like hopantenate, affects cholinergic activity through the GABAergic system.

The SART stress-induced impairment seen in the passive avoidance task was resistant to either single or repeated treatments with anxiolytic drugs, alprazolam and diazepam. The passive avoidance task has been shown to induce behavioral suppression by an adverse stimulus (punishment), resulting in a conflict situation (25). Therefore, as future problems, we feel it would be useful to evaluate the effects of benzodiazepines on retention and retrieval in addition to acquisition in the learning and memory processes of the passive avoidance task by modifying the administration schedules including administration shortly after the training trial or before the test trial.

In any case, interpretation of the data reported herein will require additional studies using other experimental situations including the shuttle-box active avoidance task in addition to evaluation of the effect of drugs affecting the cholinergic, catecholaminergic and other systems in the passive avoidance paradigm.

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