Subsensitivity to Substance P in SARI-Stressed Mice

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Abstract—The sensitivity of SARI-stressed (repeated cold-stressed) mice to substance P (SP) was studied. The behavioral responses to intrathecal SP in the stressed mice were less marked than those in non-stressed mice. The 50% effective dose of SP, however, did not differ between the 2 groups. Also, the antagonistic effect of intrathecal [D-Pro2, D-Trp7,9]-substance P against SP was not different. These results suggest that SARI-stressed mice are subsensitive to exogenous SP and that the number but not the affinity of SP receptors may be altered.

In contrast to the many reports on stress-induced analgesia, there are comparatively few reports on stress-induced hyperalgesia (1–3). In these reports, the hyperalgesia produced by exposure to a novel environment or inescapable holding (1) or exposure to horizontal oscillations (2) was described as temporary, whereas SART (specific alternation of rhythm in temperature) stress-induced hyperalgesia (3) was lasting (4) and associated with reduced tone in the sympathetic nervous system (5). SART-stressed animals are pathologically diseased and show symptoms of vagotonic-type dysautonomia; and furthermore, it may be said that the SART-stressed animal is an animal model of chronic pain.

Substance P (SP) has been proposed as a neurotransmitter for primary nociceptive afferent fibers. In behavioral studies, mice given intracerebral, intracranial or intrathecal (i.th.) SP (6, 7) show the specific behavior characteristic of irritation, which is accepted to be the result of noxious stimuli caused by SP. The purpose of our present work was to study the mechanism of SART stress-induced hyperalgesia; and in this experiment, we examined the response of SART-stressed mice to exogenously applied SP and an analogue of SP, which was reported to show SP-antagonistic effects in some experiments (8), in comparison with that of non-stressed mice.

Male ddY mice weighing about 25 g were used.
For SART stress loading, the mice were kept alternately at 24°C and 4°C at 1-hr intervals from 09:00 to 16:00 and then at 4°C from 16:00 until 09:00 the following morning. This procedure was repeated for 5–7 consecutive days (9) and stopped on the morning of the 6th or final day of stress. The stressed animals were subjected to experiments 1 hr or more after the cessation of stress.

Substance P (SP, Peptide Institute, Inc.) and [D-Pro2, D-Trp7,9]-substance P (DPDT, Peptide Institute, Inc.) were used, and these drugs were dissolved in physiological saline. In order to administer the drugs directly into the spinal subarachnoid space, mice were given an i.th. injection by lumbar puncture, introducing the needle (30 gauge) into an intervertebral space approximately at the level of the 5th and 6th lumbar vertebrae, according to the method of Hylden and Wilcox (10). The injection volume was 5 μl/mouse.

The action of SP on mouse behavior was observed according to the method described by Hylden and Wilcox (7). Mice receiving i.th. SP showed characteristic behaviors consisting of bitings, lickings and scratchings of the abdomen and hind portion of the body. The behaviors reached a peak at about 1 min after the injection and infrequently appeared 2 min or more later. Then, the intensity of the action was quantified according to the total...
number of such behaviors during a 2-min counting session from immediately after the injection.

The nociceptive threshold of mice was determined by the modified tail pressure method using a Randall-Selitto Analgesy-Meter (Ugo Basile). Force was applied to the tail at a point 1 cm distant from the root and increased at a constant rate of 16 g/sec. The force required for a mouse to produce an escape reaction was defined as the nociceptive threshold. The ratio of this force after treatment to that before treatment was defined as the antinociceptive index.

The regression line was calculated by the method of least squares. Statistical analysis was performed by Student's t-test for unpaired data. The difference between means was considered statistically significant when P<0.05.

All procedures were performed in consideration of the spirit, recommendations and policies of the guidelines for the breeding, care and use of experimental animals (the Prime Minister's Office of Japan, 1980).

The total counts of behavioral responses induced by SP were dose-related in nonstressed mice and also in SART-stressed mice, as shown in Fig. 1a. The total responses reached a maximum at the SP dose of 200 ng/mouse, in both groups. However, the total responses in SART-stressed mice were smaller than those in nonstressed mice at any dose of SP.

In Fig. 1b, total responses were expressed as a percentage response, taking the average count of total responses at 200 ng SP/mouse, a dose that induced the maximum response, which was designated as 100% in each group. The two dose-response curves overlapped with each other, and the dose producing a 50% response did not differ between nonstressed and SART-stressed mice.

Figure 2a shows the antinociceptive effect of i.th. DPDT. At higher doses, the effect was smaller in SART-stressed mice than in nonstressed mice. On the other hand, the inhibitory effect of DPDT on the behavioral response induced by i.th. administration of SP at 30 ng/mouse was much the same in both groups, as shown in Fig. 2b.

It is reported that the pain threshold to noxious chemical and thermal stimuli is dependent upon the levels of SP in the spinal cord (11) and that release of SP in the spinal cord is inhibited by noradrenaline (12). Also, the SP level in lumbar but not cervical spinal fluid from patients suffering from lower back and leg pain due to arachnoiditis has been reported to be significantly higher in comparison with that from pain-free patients (13). Yasphal et al. (14) suggested that patients with chronic pain accompanied by organic disorders might be in a state where SP is excessively released, and that other patients with chronic pain associated with nerve...
damage may be supersensitive to SP at its receptors. Of course, SART-stressed mice do not have the traumatic neural damage described by Yasphal et al. The decreased nociceptive threshold in SART-stressed animals may be due to reduced tone in the sympathetic nervous system (5). In the present study, the response to exogenous SP of SART-stressed mice was decreased. These facts suggest that in SART-stressed mice, release of SP in the spinal cord may be accelerated due to the decrease in activity of the noradrenergic system, resulting in possible subsensitivity or desensitization of these mice to the exogenous SP. This concept is consistent with a report that the hyperalgesic effect of SP was abolished by repeated i.th. injections of SP (15).

Antinociceptive effect of DPDT in SART-stressed mice was less only in comparatively larger doses than that in non-stressed mice. The change in the dose-response curve for the antinociceptive effect of DPDT caused by SART stress (Fig. 2a) was unlike the change in that for the effect of SP (Fig. 1a). DPDT has been reported to be equally effective in blocking the scratching induced by some tachykinin agonists (16), and the existence of a single population of non-interacting binding sites for tachykinin agonists has also been reported (17). Thus, the above-mentioned effect of DPDT may be due to antagonism against some tachykinin agonists in the spinal cord.

The behavioral response in SART-stressed mice produced by SP was less marked than that in non-stressed mice, but the 50% response dose did not differ between the two groups. Also, the antagonistic effect of DPDT against SP was not different between the two groups, although the antinociceptive effect of DPDT was slight in SART-stressed mice in comparison with non-stressed mice. These findings suggest that in SART-stressed mice, the affinity to SP may not be changed, but that in order to cope with the situation of accelerated release of SP, the number of its receptors may be decreased, though it is the subject for a future study to examine the quantity of SP receptors.

**References**


4 Hata, T., Kita, T., Oyama, R., Itoh, E., Kawabata,
A. and Nishimura, Y.: On the hyperalgesia in SARI-stressed mice with vagotonic-type dysautonomia. Neurosciences (Kobe) 12, 166–167 (1986) (Abs. in English)


