Characterization of Socio-psychological Stress-Induced Antinociception in the Formalin Test in Mice

Masakatsu Takahashi[1] and Hiroshi Kaneto[2]

[1]*Department of Pharmacoinformatics, School of Pharmaceutical Sciences, Nagasaki University,*
[2]*Emeritus Professor, Nagasaki University,*
1–14, Bunkyo-machi, Nagasaki 852–8521, Japan

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ABSTRACT—The antinociceptive effect induced by exposure to socio-psychological (PSY) stress using a communication box was assessed by the formalin test in mice, compared with those by exposure to footshock (FS) stress and forced swimming (SW) stress. After the termination of stress exposure, whereas exposure to FS- and SW-stress resulted in the attenuation of the formalin-induced biphasic pain response over 15 min, no appreciable antinociceptive effect was found in the case of PSY stress. When exposure to PSY stress was started during the period of early or late phase of pain after the formalin injection, the antinociceptive effect was maintained for 5–15 min; however, further exposure to PSY stress was not effective for producing antinociception. In the tail-pinch test, likewise, exposure to PSY stress longer than 5 min rather decreased the intensity of antinociception. We conclude that PSY stress in this tonic pain paradigm produces antinociception, but further continuous exposure to the emotional stress caused mice to become recuperative even in such a fear-inducing situation.

Keywords: Stress-induced analgesia (SIA), Socio-psychological stress, Formalin test, Emotional stress, Communication box

An analgesia evoked in animals by a variety of stressful events, not only a direct painful stimulus but also emotional situations by exposure to a context in which unsignaled shock was previously received (1, 2), being responsive or sympathetic to the emotional states of conspecifics (3), or encounter with natural predators (4, 5), seems to be an adaptive response of the animal to the aversive situation (stress-induced analgesia, SIA). Thus environmental stimuli arouse a fear and anxiety motivational system that is characterized by analgesia, as shown in those induced by emotionality. In our previous report (6), we demonstrated that the exposure of mice to socio-psychological (PSY) stress using the communication box produces a short-lasting antinociceptive effect in the tail-pinch and tail-flick methods.

Since the formalin test (7, 8), as an assessment of pain sensitivity, does not restrict the subject's movement, it possesses an advantage over other commonly used tail-pinch and tail-flick analgesic tests. This quality also provides an assessment of antinociception without discontinuing the stress exposure for testing. Thus the formalin test, mimicking some features of the pain that follows naturally occurring tissue damage and injury, is a more adequate model of clinical pain than the more typically used analgesic tests (9).

Because the intensity of the effect was less than that induced by footshock (FS)- and forced swimming (SW)-stress exposure, it is of interest, however, to examine whether such a lower intensity of PSY stress-induced antinociception is attributable to the duration of stress exposure or whether the analgesia by such environmental stimuli rapidly attenuates even though being persistently exposed to the stress, as an adaptation phenomenon. It is reported that in experiments of conditioned suppression, animals show a depression of response during the pain response of adjoining animals but this depression is rapidly adapted to (10, 11). In this context, we attempted to examine the nociceptive consequence of exposure to an emotional response.

MATERIALS AND METHODS

Animals

Male ddY strain mice weighing 18–20 g (Otsubo Exp.

Animals, Nagasaki) were purchased, and after reaching 23–26 g, they were employed for the experiments. All procedures used in this study were approved by the University Animal Care and Use Committee.

Stress exposure

With a modification of the method of Ogawa and Kuwahara (12), the communication box (30×30×30 cm), which consists of 9 compartments (10×10×30 cm), was used for exposure to footshock stress (FS stress) or to socio-psychological stress (PSY stress). Animals in the compartment were exposed to an inescapable and unsignaled FS (2 mA, 1-sec duration, 0.2 Hz) through the floor grid for 15 min (FS stress). Animals placed in a compartment in which the floor was covered with a plastic plate were prevented from receiving the shock, but they were exposed to PSY stress by watching and hearing the struggling, jumping and vocalization of the shocked animals for 5 min (10, 11). For SW stress, mice were forced to swim in a water bath at 20℃ for 3 min.

Formalin test

Twenty microliters of 1.0% formalin was injected subcutaneously in the dorsal surface of the right hind paw of the mouse using a microsyringe with a 26-gauge needle. The amount of time the animal spent licking or lifting the injected paw was recorded for a period of 30 min. Each mouse was individually placed in the compartment 10 min prior to the exposure to FS- and PSY-stress or the formalin injection to minimize the new environment stress. The formalin injection was performed immediately after the termination of FS-, SW- or PSY-stress exposure, and then the animals were replaced in the chamber (Experiment 1). In another experiment, mice were exposed to PSY stress immediately, 10 min and 15 min after the formalin injection up to the end of the formalin test (Experiment 2). In Experiments 1 and 2, non-stressed mice (control mice) were placed in the compartment under the some conditions as the stressed mice, and the time spent in formalin pain responses was recorded.

Tail-pinch method

The stress-induced antinociception was measured by a modified Haffner's method, the tail-pinch test. For this test, forceps of adjusted pressure were used to elicit a response time of 0.6–1.2 sec in naive animals with a cutoff time of 6 sec to avoid any damage to the tail, every 5 min from immediately after the termination of stress exposure.

Statistical analyses

The results are expressed as means±S.E.M. Following analysis of variance for repeated measures of the overall data to assess statistical significance, differences between the individual mean values in stressed- and non-stressed groups or before and after exposure to stress in each

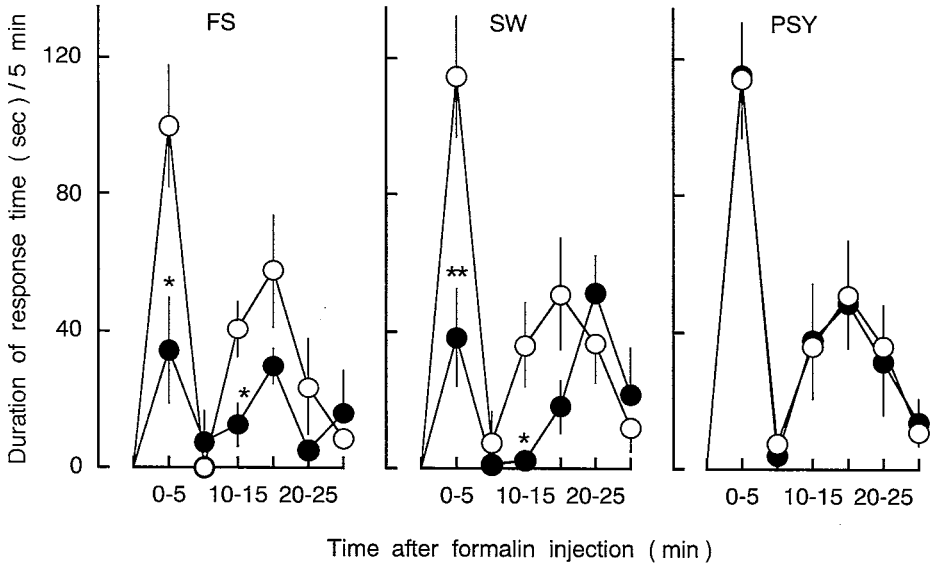

Fig. 1. Antinociceptive effect induced by exposure to footshock (FS)-, forced swimming (SW)- and socio-psychological (PSY)-stresses in the formalin test. Immediately after the termination of exposure to FS stress (left) for 15 min, SW stress (middle) for 3 min or PSY stress (right) for 5 min, mice received formalin (1.0%, 0.02 μl) subcutaneously into the dorsal surface of the right hind paw. The pain response was scored as the amount of time (sec) the animals spent licking or lifting the injected paw during a 5-min observation period. ○: non-stressed, ●: stressed. Each point represents a mean±S.E.M. *P<0.05, **P<0.01, compared with the non-stressed group for pairs of data from each 5-min period.

group are calculated by means of the unpaired Student's *t*-test for concomitant pairs of data from each 5-min period or the paired Student's *t*-test. $P<0.05$ was considered to indicate a significant difference.

RESULTS

FS-, SW- and PSY-stress-induced antinociception in the formalin test

As shown in Fig. 1, the injection of formalin produced two distinct periods of high licking activity, the early phase lasting for the first 5 min, and the late phase lasting for 15 min from 10 min after the injection. Both FS- and SW-stress resulted in the attenuation of the formalin-induced biphasic pain response over at least 15 min, while no appreciable antinociceptive effect was found in the case of PSY stress for 5 min (Experiment 1). Further exposure to PSY stress for 15 min also failed to suppress the formalin-induced pain response (data not shown).

Duration of PSY stress-induced antinociception in the formalin test

During the period of the exposure of mice to PSY stress, the antinociceptive effect, produced immediately after the start of the exposure, was maintained for 5–15 min, irrespective of the early or late phase of formalin pain. However, further exposure to PSY stress was ineffective for producing antinociception (Experiment 2, Fig. 2). Time spent in the formalin pain response for the non-stressed mice in the compartment, as shown in Fig. 2, was not substantially different from that for animals in the homecage (data not shown).

PSY stress-induced antinociception in the tail-pinch test

The exposure to PSY stress for 5, 15 and 30 min produced short-lasting antinociception in the tail-pinch method. Its intensity was maximal immediately after the termination of stress exposure and then diminished rapidly, with no appreciable analgesia observed 10 min later. However, the intensity of antinociception decreased as the duration of exposure was extended (Fig. 3).

DISCUSSION

In this report, FS- and SW-stress suppressed the formalin-induced biphasic pain response over at least 15 min, while no appreciable antinociceptive effect was found in the case of PSY stress after the termination of each stress exposure. These findings confirmed our previous report that PSY-SIA, with a peak effect immediately after the termination of stress exposure, rapidly diminished after the termination of the stress exposure in the tail-pinch and tail-flick methods (6). Accordingly, the failure of antinociception by PSY-SIA in the formalin test may be attributable to the termination of stress exposure; otherwise, it may be possible to assume that rapid

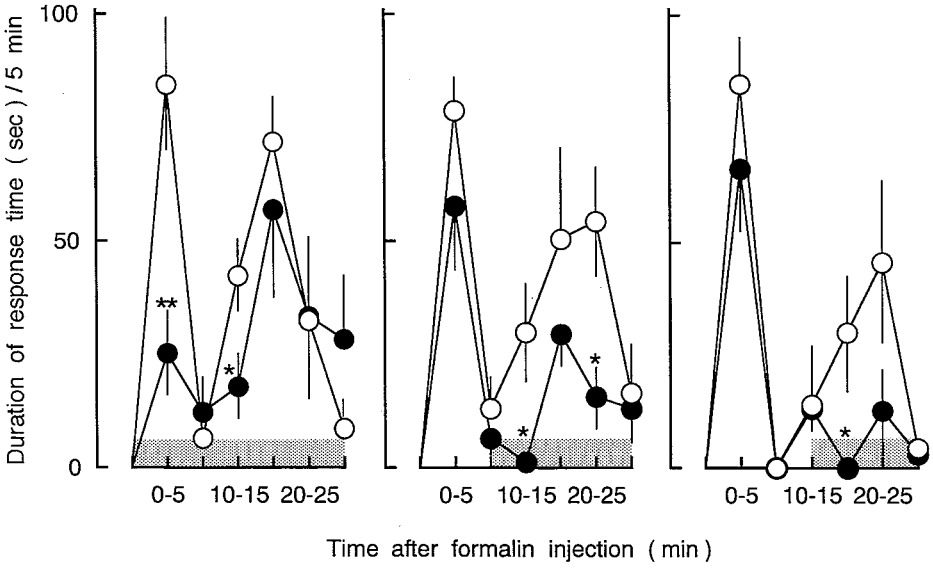

Fig. 2. Antinociceptive effect during a term of exposure to socio-psychological (PSY) stress in the formalin test. Mice received formalin (1.0%, 0.02 μl) subcutaneously into the dorsal surface of the right hind paw. PSY stress was supplied to mice immediately after (left), 10 min (middle) or 15 min (right) after the formalin injection. The pain response was scored as the amount of time (sec) the animals spent licking or lifting the injected paw during a 5-min observation period. Shaded areas indicate the term of exposure to PSY stress. ○: non-stressed, ●: stressed. Each point represents a mean±S.E.M. *$P<0.05$, **$P<0.01$, compared with the non-stressed group for pairs of data from each 5-min period.

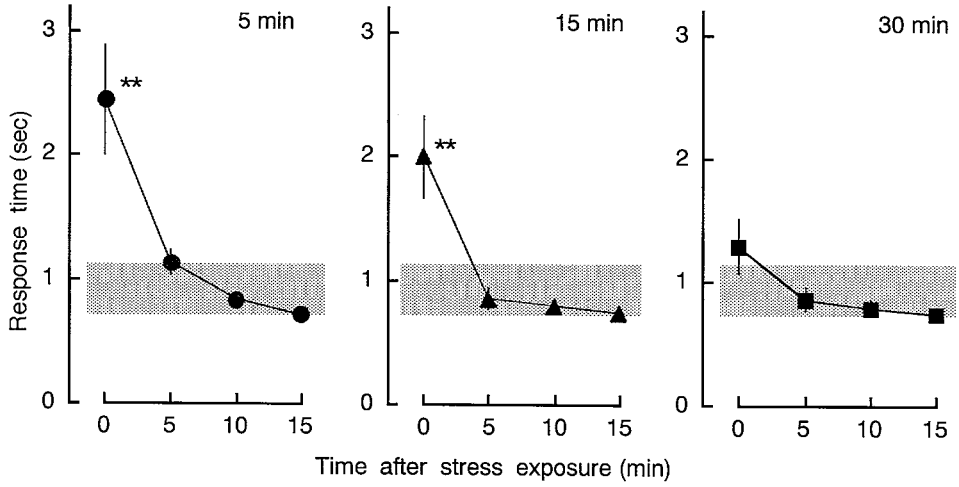

Fig. 3. Antinociceptive effect induced by exposure to socio-psychological (PSY) stress for various durations in the tail-pinch test. Mice were exposed to PSY stress for 5 min (left), 15 min (middle) or 30 min (right). The antinociceptive effect was measured by the tail-pinch method, every 5 min from immediately after the termination of stress exposure. Each point indicates the mean±S.E.M. of 8 mice. Shaded area indicates the mean±S.E.M. of the response time before exposure to stress. $^{**}P<0.01$, compared with the corresponding value before exposure to stress.

adaptation or habituation to such emotional stress easily occurs. Alternatively, PSY-SIA could be detected by the methods with phasic pain but not by the formalin test with tonic pain, partially due to the low intensity of PSY-SIA. Thus, we examined the characteristics of antinociception induced by PSY stress without discontinuation of the stress exposure during the test.

In these studies, we found that even the continuous exposure to the emotional stress caused mice to be recuperative in the aversive situation even though they were not released from such a stressful environment. Conceivably, painful exhibition by conspecifics produced analgesia in the others which are sympathetic to the victim; however, the state should be nullified by the more adaptative situation, arousing the pain alertness instead of defensive behavior to fear, even under the environment where there is a confrontation with a threating situation.

The stress-induced antinociception using the communication box rapidly diminished by daily exposure to the stress; on the contrary, animals exposed to a context previously paired with an painful event produce an analgesic effect, and this effect, designated conditioned-fear-induced analgesia, was daily enhanced (1, 13, 14), suggesting the multiplicity of activation of pain-inhibitory systems and adaptation systems by stresses.

Thus, using the formalin test, it is evident that the PSY stress possesses a suppressive effect in response to tonic pain, expressed as recuperative behaviors, e.g., paw licking in this test, as well as phasic pain expressed as withdrawal reflexes, induced by the tail-flick and tail-pinch methods (15). Furthermore, we found that the analgesic effect induced by the PSY stress becomes diminished despite of continuous exposure to the aversive stimuli, suggesting that continuous activation of the suppressive mechanism in response to painful stimuli would be disadvantageous rather than helpful in confronting emergency situations.

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