Effect of Neurotropin on Hyperalgesia Induced by Prostaglandin E2, Naloxone, Melatonin and Dark Condition in Mice

Hiroshi TAKAHASHI, Manabu SHIBATA, Tsuyako OHKUBO, Kihachi SAITO* and Reizo INOKI*

Department of Pharmacology, Fukuoka Dental College, Sawara-ku, Fukuoka 814-01, Japan  
*Department of Pharmacology, Faculty of Dentistry, Osaka University, Suita, Osaka 565, Japan

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Abstract—Subcutaneous injection of formaldehyde into mouse hind paw elicited pain responses consisting of licking or biting of the paw, which were observed biphasically. The first and second phases were enhanced by melatonin and melatonin, naloxone, prostaglandin E2, respectively. Mice kept in the dark also exhibited hyperalgesic response. When neurotropin was injected intraperitoneally 30 min prior to those treatments, hyperalgesia was suppressed to the control level. Aspirin inhibited only the second hyperalgesic phase.

Neurotropin was extracted from inflamed skin of rabbit inoculated with vaccinia virus based on the idea that compulsory inflammation may produce endogenous materials preventing inflammation and pain sensation (1, 2) and is clinically used as an analgesic with few side effects. This idea is also compatible with the phenomena involving autonolgesia induced by many types of noxious stimuli. Analgesic activity of neurotropin was shown in SART-stressed mice, whereas no effect was observed in non-stressed mice (3). The lower threshold level of pain sensation was observed in SART-stressed mice (4), and neurotropin was able to increase pain threshold to the normal level. This was further supported by the report that neurotropin was more effective in patients with severe pain (5).

Quite a few reports demonstrated the development of hyperalgesia following the administration of prostaglandin (PG) E2 (6–8) or naloxone (9, 10). We have shown that melatonin produced hyperalgesic action in mice (11, 12) and that mice kept in the dark developed the hyperalgesia (11). In the present study, the effect of neurotropin on the hyperalgesia induced by those conditions was investigated with respect to involvement of endogenous materials suppressing hyperalgesia.

Male mice (ddY, 20–25 g) were used. Pain responses were measured by cumulating the time (sec) spent for licking or biting the paw observed following the subcutaneous injection of 25 µl of diluted formalin (0.5% formaldehyde solution) into right hind paw for 30 min with five minutes intervals. The method was the modification of Dubuisson and Dennis (13). Mice were kept at 25°C with a 12 hr light-dark cycle with illumination of intensity of 150–300 lux. Mice showing hyperalgesia under the dark condition were kept at the illumination intensity of 10 lux for 30 min before the injection of formalin. Also, hyperalgesic animals were obtained by intracerebroventricular (i.c.v.) injection of melatonin and PGE2 and subcutaneous injection of naloxone 20 min before formalin injection. Effect of neurotropin was examined by administering it 30 min prior to formalin injection. Naloxone was dissolved in saline solution. Melatonin and PGE2 were dissolved in 10% and 0.2% ethanol, respectively. Neurotropin (Nippon Zoki Pharm. Co.), melatonin (Sigma Chem. Co.), naloxone hydrochloride (Wako Pure Chem. Co.), and PGE2 (Nakarai Chem. Co.) were used. Significant differences were determined by Student's t-test. When formalin was injected into the hind paw, pain response consisting of licking or biting of the paw was observed biphasically.
Right after the injection of formalin, licking and biting behaviors were observed strikingly for the first five minutes (first phase). In the subsequent 5 min period, almost no licking or biting behavior was observed. They reappeared about 10 min after the formalin injection, and the maximum value was obtained from 15 to 20 min (second phase). It was thought that the first phase response might be non-inflammatory pain evoked by direct chemical stimulation of the nerve ending, and the second phase response might be due to ensuing inflammation (14). Neurotropin at the doses of 50–300 mg/kg (i.p.) affected neither the first nor second phase responses (Fig. 1).

The change of pain threshold induced by various conditions and the effects of neurotropin and aspirin were investigated (Table 1). It was found that i.c.v. injection of vehicle reduced both the first and second phase responses. One hundred ng of PGE2 (i.c.v.) enhanced the second phase pain response significantly, while it was without effect on the first phase response. When neurotropin (50 mg/kg, i.p.) was administered prior PGE2, it depressed PGE2 enhanced response without affecting the first phase response. Naloxone (2 mg/kg, s.c.) also enhanced the second phase pain response significantly. Suppressive effects of neurotropin (50 mg/kg, i.p.) and aspirin (50 mg/kg, p.o.) on the naloxone-induced hyperalgesia were observed. The same dose of aspirin had no effect in normal mice.

The previous reports (9, 10) showed that an i.c.v. injection of 2–20 μg melatonin induced hyperalgesia dose-dependently. The hyperalgesic effect of melatonin (20 μg) on formalin-induced pain response was observed for both the first and second phases, and those were suppressed by neurotropin. Aspirin suppressed only the second phase.

Hyperalgesia induced under the dark condition was inversely dependent on the intensity of illumination (11). When mice were kept in the dark (10 lux) for 30 min, formalin-induced pain responses both in the first and second phases increased. Neurotropin significantly suppressed the hyperalgesia in both the phases.

The major interest of the present study is that components derived from endogenously produced materials in inflamed rabbit skin suppress the hyperalgesia induced under various conditions. Our approach is similar to that of Ferreira et al. who employed PGE2 to induce the state of hyperalgesia and investigate the peripheral analgesic effect of opioid peptides (6, 7). Okuyama et al. also investigated the effect of analgesics on the hyperalgesia following the i.c.v. injection of arachidonic acid, PGE2 or PGF2α (8). In the present study, neurotropin appeared to be effective for suppressing hyperalgesia induced by many types of stimuli. Our results that neurotropin suppressed the hyperalgesia is consistent with the previous finding that neurotropin is effective for SART-stressed mice (3) wherein the low level of pain threshold is reported (4). Its universal effect in hyperalgesia induced by different conditions suggests the common feature, at least in part, of those pathological states. Neurotropin is different from aspirin in the site of action, since aspirin suppressed the hyperalgesia only in the second phase. Bradykinin may be involved in the aforementioned phenomena, since we have previously reported that neurotropin inhibited the noxious stimuli induced release of bradykinin-like substances into the perfusate of rat hind paw (15). Substance P also may be
Table 1. Effects of neurotropin and aspirin on hyperalgesia induced by naloxone, prostaglandin E₂, melatonin and dark condition

|                     | First phase |                  | Second phase |                  |
|---------------------|-------------|-----------------|--------------|-----------------|
|                     | Control     | Hyperalgesic    | Control      | Hyperalgesic    |
|                     | animals     | animals         | animals      | animals         |
|                     | NTP (i.p.)  | APN (p.o.)      | NTP (i.p.)   | APN (p.o.)      |
| Dark condition      | 10 lux      | 90.5±14.0       | 141.0±5.0f   | 101.0±7.3***    |
|                     |             | 66.7±9.3a       | 100.0±15.0   | 51.5±6.0**      |
| Naloxone            | 2 mg/kg (s.c.) | 100.0±5.5b     | 108.0±14.0   | 104.0±9.5       |
|                     |             | 100.2±7.0       | 75.0±5.1b    | 109.1±9.9tt     |
|                     |             |                 | 59.2±5.8***  | 55.0±9.3***     |
| Prostaglandin E₂    | 100 ng/mouse (i.c.v.) | 37.7±5.5c     | 44.3±5.7     | 44.2±5.2        |
|                     |             | 35.5±4.0c       | 75.6±10.0tt  | 27.7±6.6***     |
| Melatonin           | 20 μg/mouse (i.c.v.) | 48.9±4.6d      | 67.1±3.1t    | 32.4±7.4**      |
|                     |             | 62.2±2.3        | 29.5±6.3d    | 60.9±4.1tt      |
|                     |             |                 | 14.2±6.0***  | 13.7±6.9**      |

NTP: neurotropin, 50 mg/kg, i.p.; APN: aspirin, 50 mg/kg, p.o. Each value indicates the duration of pain response (in sec) and presents the mean±S.E. (n=7). Pain response of control animals receiving vehicle (*a-b 0.9% saline, c 0.2% ethanol and d 10% ethanol were administered, respectively). *a,b: Normal control. fP<0.05, tP<0.01, tttP<0.001: P value against control animals. **P<0.01, ***P<0.001: P value against hyperalgesic animals.
involved in the suppressive effect of neurotropin, because neurotropin inhibited neurogenic inflammation in which substance P takes part (M. Shibata, personal communication).

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