Differential Effects of Whole-body γ-Irradiation on Antinociception Induced by Morphine and β-Endorphin Administered Intracerebroventricularly in the Mouse

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ABSTRACT
Two separate lines of evidence suggested the present study. First, intracerebroventricularly (i.c.v.) administered morphine (a µ-opioid receptor agonist) and β-endorphin (an ε-opioid receptor agonist) produce antinociception by activating different descending pain inhibitory systems. Second, γ-irradiation attenuates the acute antinociceptive action of i.c.v. injected morphine, but not DPLPE (a δ-opioid receptor agonist), in mice. These findings prompted us to investigate the effect of γ-irradiation on the antinociception produced by i.c.v. injected morphine and β-endorphin in male ICR mice. In one group, mice were exposed to whole-body irradiation at a dose of 5 Gy from a 60Co γ-source and the antinociceptive effects were tested 5, 30, 60, 90 and 180 min after irradiation using the 1 % acetic acid-induced writhing test (10 ml/kg). The antinociceptive effect was produced time-dependently and reached its maximum at 90 min after irradiation. Thus, time was fixed in the following studies. In another group, mice were irradiated with 5 Gy and tested 90 minutes later for antinociception produced by i.c.v. administration of morphine (50 and 100 ng/mouse) or β-endorphin (31 ng/mouse). Irradiation significantly potentiated the antinociception produced by β-endorphin. However, the antinociception produced by morphine was not affected by irradiation. These results demonstrate a differential sensitivity of µ- and ε-opioid receptors to γ-irradiation, in addition, support the hypothesis that morphine and β-endorphin administered supraspinally produce antinociception by different neuronal mechanisms.

Keywords: Antinociception, β-Endorphin, Morphine, γ-Irradiation, Mouse

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INTRODUCTION
It is well established that different forms of stress, such as cold water swimming, immobilization, and whole body irradiation (WBI), produce a pronounced antinociception in experimental animals (1,2,3). Some studies have suggested that WBI-induced antinociception is mediated through opioid receptors (2,4,5). Indeed, a WBI with 2.5-15 Gy produced an antinociception which was significantly reduced by naloxone, a nonselective opioid receptor antagonist in the hot-plate test (5).

It has been reported that β-endorphin, an ε-opioid receptor agonist, produces its antinociceptive response when injected into the brain, and that the pharmacological and biochemical actions of β-endorphin are different from those of morphine, a µ-opioid receptor agonist (6,7). A differential sensitivity of µ- and δ-opioid receptors to WBI (2) can still be possible.

The aim of the present study was to determine the effect of WBI stress on the antinociceptive action of β-endorphin and morphine administered intracerebroventricularly. Antinociception was assessed in male ICR mice using the 1% acetic acid-induced abdominal constriction (writhing) test.

METHODS
Animals: Male ICR mice (Korean Chemical Research Institute, Daejon, Korea) weighing 26-27 g were used for all the experiments. Animals were housed 5 per cage in a room maintained at 22 ± 0.5 °C with an alternating 12 hour light-dark cycle for at least 5 days before the experiments were started and food and water were available ad libitum. Each animal was used only once. These experiments were approved by the Korean Laboratory Animal Care and Use Committee. All procedures were conducted in accordance with the ethical guidelines of the International Association for the Study of Pain. All antinociceptive measures were recorded between 11:00 and 14:00 h.
Whole body Irradiation: Mice were whole body irradiated in the unilateral γ-radiation field in Korea Atomic Energy Research Institute (KAERI) 60Co facility (Source strength 150 TBq, Panoramic Irradiator, Atomic Energy of Canada Ltd.). During irradiation, the animals were confined in a well-ventilated Plexiglas box (10 x 3.5 x 4 Cm). Five grey (Gy) were delivered at a dose rate of 167 cGy/min. Prior to the irradiations of the animals, the dose rate was determined by a Fricke dosimeter (8). Sham-irradiated control mice were treated similarly to the irradiated mice, except that the 60Co source elements were not raised into the exposure positions.

Assessment of antinociception: The pain sensitivity of mice was measured by the acetic acid induced abdominal constriction (writhing) test. For the writhing test, the control or treated mice were gently placed in individual Plexiglas box (4 x 7 x 15 Cm) and the writhing numbers were counted at 5-min intervals for 20 minutes. The counting of abdominal constrictions was started immediately after the injection of acetic acid (1%, 0.1 ml/10g body weight) intraperitoneally. A decrease in writhing numbers was used as a marker of antinociception.

Intracerebroventricular (i.c.v.) injection: I.c.v. injections were made according to the procedure of Haley & McCormick (9). The i.c.v. injection volume was 5 µl and the injection site was verified by injecting a similar volume of 1% methylene blue solution and determining the distribution of the injected dye in the ventricular space. The dye injected i.c.v. was found to be distributed through the ventricular spaces and reached the ventral surface of the brain and the dye was found in the upper cervical portion of the spinal cord. The experiments were trained, using an injection of dye in the beginning of the experiments, to achieve a 95% or more accuracy for i.c.v. injections in other mice.

Experimental protocol: Mice were whole body irradiated with γ-ray at a dose of 5 Gy and after 90 minutes the writhing test was carried out. β-endorphin (31 ng) and morphine (50 or 100 ng) were administered intracerebroventricularly 10 minutes prior to the writhing test.

Drugs: The drugs used in the present experiment were β-endorphin (Research Biochemicals Inc., Natick, MA) and glacial acetic acid (Sigma). These drugs were dissolved in a sterile saline (0.9 % NaCl) solution.

Statistical analyses: The significance of changes in antinociceptive responses was assessed by an analysis of variance with repeated measures and after significant interaction this was followed by the Student-Newman-Keuls test.

RESULTS

The antinociceptive effect was produced time-dependently and reached its maximum at 90 min after whole body irradiation with 5 Gy of γ-ray (Fig. 1). Even though the antinociception produced by β-endorphin (i.c.v., 31 ng/mouse) continued for more than 20 minutes, it reached its maximum at 10 min after injection (Fig. 2). To analyze the effect of whole body irradiation on the β-endorphin (EP)-induced antinociception, mice were whole body irradiated with γ-ray and β-endorphin was administered 10 minutes before the writhing test. Writhing numbers were counted at 90 min after irradiation when the radiation-induced antinociception reached its maximum. The writhing numbers were greatly reduced in the WBI+EP-treated group (Fig. 3). The antinociceptive effect induced by irradiation and EP injection was significantly higher than that in the sham+EP-treated group (p < 0.005). It was confirmed that the antinociception induced by β-endorphin was potentiated by whole body irradiation. However, the antinociception produced by morphine (50 and 100 ng/mouse) was not affected by whole body irradiation. (Fig. 4). These results demonstrate the differential sensitivity of µ- and ε-opioid receptors to γ-irradiation.

DISCUSSION

The antinociceptive potency of opioids may be influenced by various stresses. In most instances, the exposure of experimental animals to a variety of stressful manipulations initiates increases in baseline nociceptive thresholds, but reduces the ability of a narcotic to produce an antinociceptive response. The findings of the present study demonstrate that exposure to WBI stress produces an antinociception in the acetic acid-induced writhing test, suggesting that WBI stress also produces an antinociception in the acetic acid-induced writhing test. Previous studies have reported that morphine-induced antinociception was attenuated by WBI
stress. We have also recently found in the tail-immersion test that morphine given i.c.v. produced an antinociception and that this effect was attenuated following the exposure to WBI stress (unpublished data). However, in the present study the morphine (i.c.v.)-induced antinociception failed to be changed by WBI stress. This discrepancy in the effect of WBI against the morphine-induced antinociception between in the thermal tail-flick test and in the acetic acid induced writhing test is presently unknown, but may be due to the difference of sensitivity of both tests to morphine. Indeed, based on our and other’s results, the acetic acid-induced writhing test is the more sensitive pain model than the thermal tail-flick test and thus needs a lesser dose of morphin to produce the same level of antinociceptive effects. As an alternative explanation to this result, it could be due to the modality difference between thermal and chemical noxious stimuli. However, we can not exclude the other possibility.

We found in the present results that β-endorphin (i.c.v.)-induced antinociception was not attenuated, but potentiated, in the acetic acid-induced writhing test following the WBI stress. Although these data are very interesting, we can not explain the exact mechanism responsible for the development of the increase in the antinociceptive responsivity to β-endorphin. It has been known that morphine and β-endorphin given i.c.v. binds µ- and ε-opioid receptors and produces their antinociceptive effects through a different action mechanism. Furthermore, Raffa et al. (2) reported that µ-, but not δ-, opioid receptor-mediated antinociception in mice is attenuated by WBI stress. Taken together, it is likely that WBI stress has a differential sensitivity against the antinociceptive effect produced by stimulation each subtype of opioid receptor.

In summary, the antinociceptive effect was produced time-dependently and reached its maximum at 90 min after whole body irradition with γ-ray (5 GY). In addition, the antinociceptive effect produced by β-endorphin (i.c.v.) was potentiated whereas that of morphine was not affected by the WBI stress. These results demonstrate a differential sensitivity of µ- and ε-opioid receptors to WBI stress, and, in addition, support the hypothesis that morphine and β-endorphin administered supraspinally produce antinociception by different neuronal mechanisms.

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REFERENCES

Figure 1. Time course of changes in the writhing numbers after whole body irradiation. Mice were whole body irradiated with 5 Gy of γ-ray and allowed to rest for the indicated intervals before the writhing test. Writhing numbers were counted before, 5, 30, 60, 90 and 180 minutes after WBI. The data are mean ± SEM (n = 8). *p < 0.05; **p < 0.01, significantly different from that counted before WBI.

Figure 2. Time course of changes in the writhing numbers after the injection of β-endorphin (EP). Mice were treated with EP (i.c.v.) at a dose of 31 ng in the writhing test and allowed to rest for the indicated intervals before both tests. Writhing numbers were counted before 5, 10 and 20 minutes after i.c.v. EP injection. The data are mean ± SEM (n = 8). *p < 0.05; **p < 0.01, significantly different from that counted before EP injection.
Figure 3. The effect of whole body irradiation on the β-endorphin (EP)-induced antinociception in the writhing test. Mice were whole body irradiated with 5 Gy of γ-ray and β-endorphin (31 ng, i.c.v.) was administered 10 minutes before the writhing test. Writhing numbers were counted 90 minutes after WBI. The data are mean ± SEM (n = 8). **p < 0.01; ***p < 0.005, significantly different from that of control group (no treatment); +++p < 0.005, significantly different from that of sham+EP-treated group.

Figure 4. The effect of whole body irradiation (WBI) stress on the morphine-induced antinociception in the writhing test. Mice were whole body irradiated with 5 Gy of γ-ray and morphine (50 or 100 ng, i.c.v.) was administered 10 minutes before the writhing test. Writhing numbers were counted 90 minutes after WBI. The data are mean ± SEM (n = 8).