International Journal of Oral Biology, Vol. 34, No. 3, September 30 2009, p. 137~142

International Journal of Oral Biology

The Differential Effect of Whole-body Irradiation on Morphine- and β-Endorphin-Induced Antinociceptive Actions in Mice

Kyung N. Kim and Ki M. Chung*

Department of Physiology and Neuroscience, College of Dentistry and Research Institute of Oral Science, Gangneung-Wonju National University, Gangneung, 210-702, Korea

(received September 8, 2009 ; revised September 14, 2009 ; accepted September 18, 2009)

Whole-body γ-irradiation(WBI), which produces an oxidative stress, is reported to attenuate the acute antinociceptive action of morphine (a μ-opioid receptor agonist), but not DPLPE (a δ-opioid receptor agonist), in mice. Recently, we also reported that antinociceptive effect of morphine, but not β-endorphin (a novel ε-opioid receptor agonist), was attenuated by oxidative stress. These findings prompted us to investigate the effect of WBI on the antinociception of morphine and β-endorphin in mice. Mice were exposed to WBI (5 Gy) from a ^{60}Co gamma-source and tested 2 hours later for antinociception produced by intracerebroventricular administration of morphine or β-endorphin using the hot water tail-immersion and the writhing tests. WBI significantly attenuated the antinociception produced by morphine only in the hot water tail-immersion test, whereas the antinociception of β-endorphin was significantly potentiated by WBI in both tests. These results demonstrate a differential sensitivity of μ- and ε-opioid receptors to WBI, and support the hypothesis that morphine and β-endorphin administered supraspinally produce antinociception by different neuronal mechanisms.

Key words: Antinociception, β-Endorphin, Morphine, Whole-body γ-irradiation, Mouse

*Corresponding author: Ki-Myung Chung, Ph.D., Associate Professor, Department of Physiology and Neuroscience, College of Dentistry & Research Institute of Oral Science, Gangenung-Wonju National University, 120 Gangneung Daehangno, Gangneung, 210-702, Korea. Tel.: +82-33-640-2461; Fax.: +82-33-642-6410; E-mail: ckm@gwnu.ac.kr

Introduction

People and animals are often faced in their environment with a variety of stresses. An exposure of rats or mice to stressful circumstances, such as swimming, electric footshock, immobilization, noise, nociceptive stimuli or whole-body irradiation (WBI), produces a decrease in pain sensitivity, termed stress-induced analgesia (Watkins and Mayer, 1982; Terman *et al.*, 1984; Raffa *et al*., 1988; Mahajan *et al.*, 1997; Suh *et al*., 1999; Ahn *et al.*, 2008). Some studies have suggested that WBI-induced antinociception is mediated through opioid receptors (Mickley *et al*., 1984; Teskey and Kavaliers, 1984; Raffa *et al*., 1988). 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 (Teskey and Kavaliers, 1984).

Among the opioid receptors, which have been pharmacologically classified as μ, δ, κ, and ε, the existence of the ε-opioid receptor has been controversial, and this receptor is generally not recognized as a member of the opioid receptor family because it has not been precisely characterized. However, results from pharmacological, physiological and opioid receptor binding studies clearly indicate the presence of ε-opioid receptors, which are distinct from μ, δ, or κ-opioid receptors. This putative ε-opioid receptor is stimulated supraspinally by the endogenous opioid peptide β-endorphin, which induces the release of Met-enkephalin, which, in turn, acts on spinal and trigeminal δ2-opioid receptors to produce antinociception (Jung *et al*., 1989; Suh *et al.*, 1989; Chung *et al*., 1997, 2003; Chung and Suh, 2001; Tseng, 2001; Seo *et al*., 2008). Meanwhile, the classical opiate morphine is widely used in the clinical management of severe acute and chronic pain and shows a preference for μ-opioid receptors.

A previous study demonstrated that a differential sensitivity of μ- and δ-opioid receptors to WBI (Raffa *et al*., 1988). Recently, we also reported that oxidative stress, which was produced by depletion of brain glutathione level, has a differential modulatory effects on the antinociceptive effects produced by morphine and β-endorphin (Chung *et al.,* 2003). Thus, it might be very interesting to investigate the effect of WBI on the antinociception of morphine and β-endorphin in mice.

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. The pain sensitivity of mice was assessed by the hot water tail-immersion and the 1% acetic acid-induced abdominal constriction (writhing) tests.

Methods

Animals

Male ICR mice (Daehan Lab., 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. All experimental procedures were conducted under protocols approved by IACUC (Institutional Animal Care and Use Committee) of Gangneung-Wonju National University, and were performed between 11:00 and 14:00 h.

Whole-body Irradiation (WBI)

Mice were whole-body irradiated in the unilateral γ-radiation field in the Korean Atomic Energy Research Institute (KAERI) ^{60}Co facility (Panoramic Irradiator, approximately 1000 Ci capacity, Atomic Energy of Canada Ltd.). During irradiation, the animals were confined in well-ventilated Plexiglas box (10 × 3.5 × 4 Cm). 5 Gy were delivered at a dose rate of 167 cGy/min. Prior to irradiations of the animals, the dose rate was determined by Fricke dosimeter (Niels and Roger, 1970). Sham-irradiated control mice were treated similarly to the irradiated mice, except that the ^{60}Co source elements were not raised into the exposure positions.

Assessment of antinociception

For the writhing test (Koster *et al*., 1959), mouse was administered intraperitoneally with 0.5 ml of 1% acetic acid dissolved in saline. The number of writhes was counted during a 20 min period following the injection of acetic acid. A writhe was defined as a contraction of the abdominal muscles accompanied by an extension of the forelimb and elongation of the body.

For the hot water tail-immersion test (Ben-Bassar *et al*., 1959; Chung *et al.*, 2003), the temperature of the hot water was maintained at the temperature of 52 ± 0.1°C. The tail withdrawal latencies were determined by placing the distal part of the tail (5 Cm) in a plastic beaker. Antinociceptive effects were presented as percentage of MPE (% MPE), which was calculated as $[(T_1 - T_0) / (10 - T_0)] \times 100$, where T_0 and T_1 were the tail-flick latency time before and after treatment, respectively.

Intracerebroventricular (i.c.v.) injection of morphine and β-endorphin

I.c.v. injections were made according to the procedure of Haley and McCormick (1957). 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. Each of morphine and β-endorphin was administered 10 min prior to the writhing test, and the doses used in the test were 50 ng/5 μl for morphine and 31 ng/5 μl for β-endorphin, respectively. In the hot water tail-immersion test, the antinociceptive effects were tested 120 min following WBI, and morphine and β-endorphin were injected 20 min for morphine and 30 min for β-endorphin, respectively, prior to the test. The doses of morphine and β-endorphin used were 0.2-1 μg/5 μl and 1 μg/5 μl, respectively.

Drugs

Morphine (Jeil Pharmaceutics, Korea) and β-endorphin (Research Biochemicals Inc., Natick, MA) were dissolved in a sterile saline (0.9% NaCl) solution. Other drugs were purchased from Sigma Chemical Co. (St. Louis, Mo).

Statistical analyses

The significance of changes in antinociceptive responses was assessed by analysis of variance with repeated measures and followed by the Student-Newman-Keuls test.

Results

The effects of whole-body irradiation on the inhibition of the acetic acid-induced writhing responses induced by i.c.v. β-endorphin or morphine

After WBI, mice were subjected for the writhing test. As shown in Fig. 1, WBI at a dose of 5 Gy produced the inhibition of the writhing response. The maximal antinociceptive effect

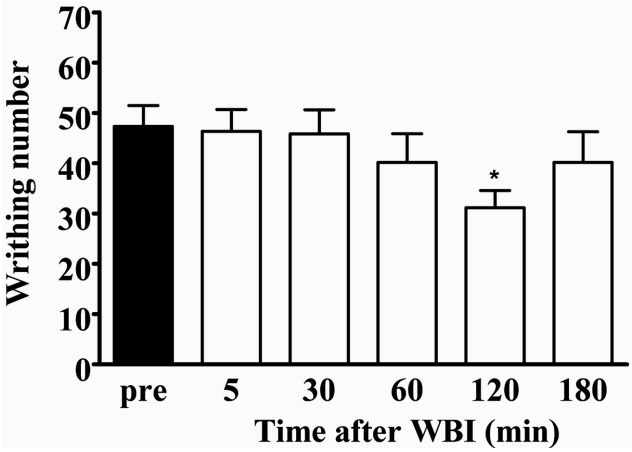

Fig. 1. Time course of change in the writhing numbers after whole body irradiation (WBI) stress. Mice were whole body irradiated with 5 Gy of γ-ray and allowed to rest for the indicated intervals (5, 30, 60, 120 and 180 mins after WBI) before the writhing test. The number of writhing was counted for 20 min following the intraperitoneal injection of 1% acetic acid solution dissolved in saline. The data are mean ± S.E.M. (n = 8). *p < 0.05, vs. Pre(control) group.

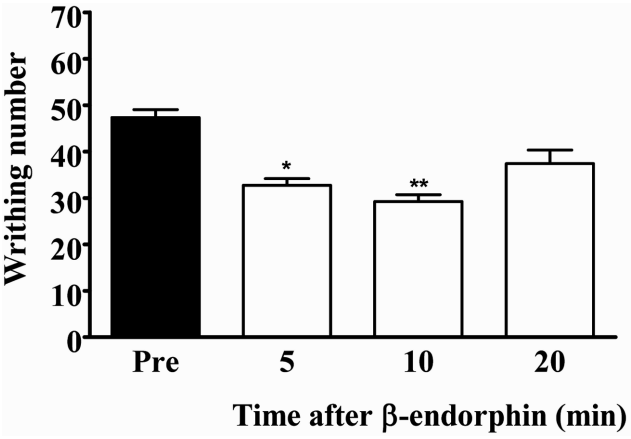

Fig. 2. Time course of changes in the writhing numbers after the injection of β-endorphin (i.c.v.). Mice were administered with β-endorphin (i.c.v.) at a dose of 31 ng, and allowed to rest for the indicated intervals before the writhing test. Writhing numbers were counted before, and 5, 10 and 20 min after the β-endorphin(i.c.v.) injection. The data are mean ± S.E.M. (n = 8). *p < 0.05 and **p < 0.01 vs. Pre(control) group, respectively.

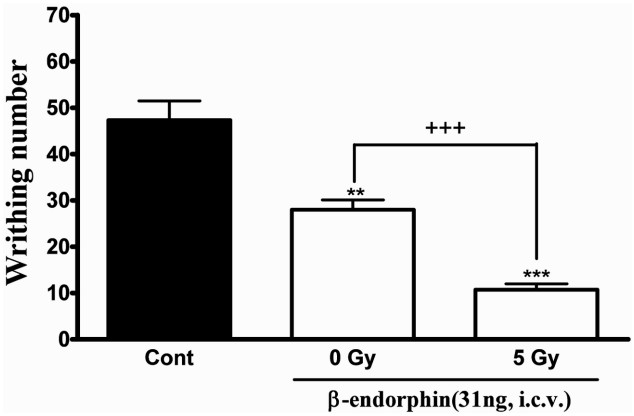

Fig. 3. The effect of whole body irradiation (WBI) stress on the β-endorphin (i.c.v.)-induced antinociception in the writhing test. Mice were whole body irradiated with 5 Gy of γ-ray, and allowed to rest for 120 min before the writhing test. β-endorphin (31 ng, i.c.v.) was administered 10 min before the writhing test. The writhing numbers were counted for 20 min. The data are mean ± S.E.M. (n = 8). **p < 0.01 and ***p < 0.005, vs. Cont group (no treatment), respectively, $^{+++}$p < 0.005, vs. sham treatment+β-endorphin group.

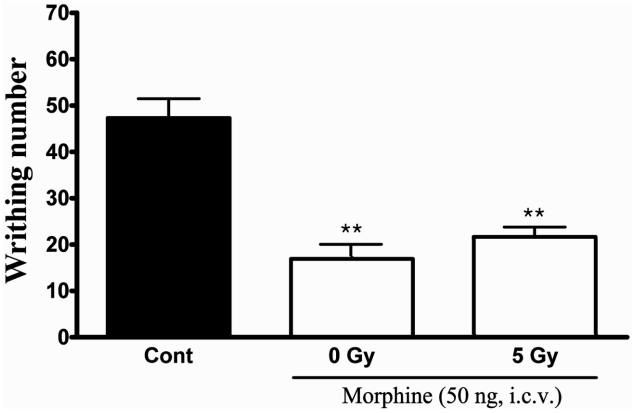

Fig. 4. The effect of whole body irradiation (WBI) stress on the morphine (i.c.v.)-induced antinociception in the writhing test. Mice were whole body irradiated with 5 Gy of γ-ray, and allowed to rest for 120 min before the writhing test. Morphine (50 ng, i.c.v.) was administered 10 min before the writhing test. The writhing numbers were counted for 20 min. The data are mean ± S.E.M. (n = 8). **p < 0.01, vs. Cont group (no treatment).

was shown at 120 min after WBI, and thereafter declined slowly. Thus, every of the following writhing studies were assessed at 120 min after WBI. Next, β-Endorphin was administered i.c.v. in a dose of 31 ng per mouse. Then, the writhing response was measured at 5, 10 and 20 min after β-endorphin administration. As shown in Fig. 2, the maximal antinociceptive effect was shown at 10 min after β-endorphin administration and thereafter declined slowly. Then, the antinociceptive effects of morphine and β-endorphin were examined in sham- or WBI-treated mice. The antinociceptive effects were measured using the writhing test. The writhing responses were measured at 10 min following i.c.v. administrations of morphine or β-endorphin for 20 min. The i.c.v. administration of morphine (50 ng) and β-endorphin(31 ng) induced pronounced antinociceptive effects in sham-treated mice. The effect of morphine was not significantly attenuated by WBI (Fig. 4.), while that of β-endorphin was interestingly potentiated by WBI exposure (Fig. 3.).

The effects of whole-body irradiation on the inhibition of the hot water immersion tail-flick responses induced by i.c.v. β-endorphin or morphine

After WBI, mice were subjected for the hot water tail-immersion test. As shown in Fig. 5, WBI at a dose of 5 Gy

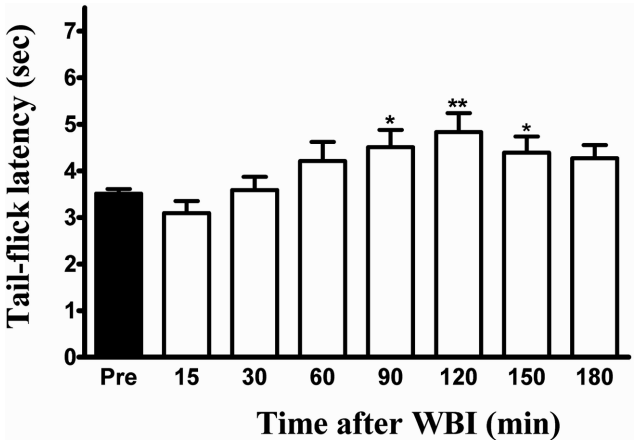

Fig. 5. Time course of change in the tail-flick latencies after whole body irradiation (WBI) stress. Mice were whole body irradiated with 5 Gy of γ-ray and allowed to rest for the indicated intervals (5, 30, 60, 120 and 180 mins after WBI) before the hot water tail-immersion test. The data are mean ± S.E.M. (n = 8). *p < 0.05 and **p < 0.01, vs. Pre group, respectively.

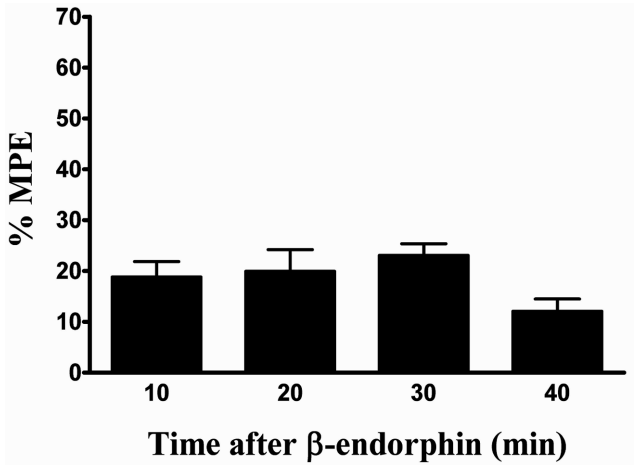

Fig. 6. Time course of changes in the tail-flick latencies after the injection of β-endorphin (i.c.v.). β-Endorphin (0.2 μg/5 μl) was injected into the mouse third ventricle 30 min prior to the hot water tail-immersion test. % MPE denotes the percentage of the maximal possible effect. The data are represented as the mean ± S.E.M. (n = 8). *p < 0.05 and **p < 0.01, vs. pre group, respectively.

produced the inhibition of the tail-flick response. The maximal antinociceptive effect was shown at 120 min after WBI, and thereafter declined slowly. Thus, all the following hot water tail-immersion studies were assessed at 120 min after WBI. Next, β-endorphin was administered i.c.v. in a dose of 0.2 μg per mouse. Then, the tail-flick latency was measured at 10, 20, 30 and 40 min after β-Endorphin administration. As shown in Fig. 6, the maximal antinociceptive effect was shown at 30 min after β-endorphin administration and thereafter declined slowly. Then, the antinociceptive effects of morphine and β-endorphin were examined in sham- or WBI-treated mice. The antinociceptive effects were measured using the hot water tail-immersion test. Morphine

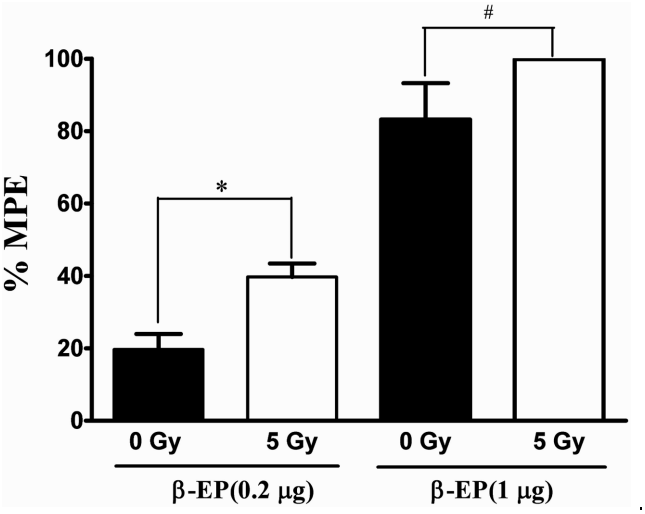

Fig. 7. The effect of whole body irradiation (WBI) stress on the β-endorphin (β-EP; i.c.v.)-induced antinociception in the hot water tail-immersion test. Mice were whole body irradiated with 5 Gy of γ-ray, and allowed to rest for 120 min. β-endorphin (0.2 μg/5 μl or 1 μg/5 μl) was injected into the mouse third ventricle 30 min before the test. % MPE denotes the percentage of the maximal possible effect. All data are represented as the means ± S.E.M. (n = 8-10). *p < 0.05 vs. sham+β-EP(0.2 μg/5 μl), #p < 0.05 vs. sham+β-EP(1 μg/5 μl).

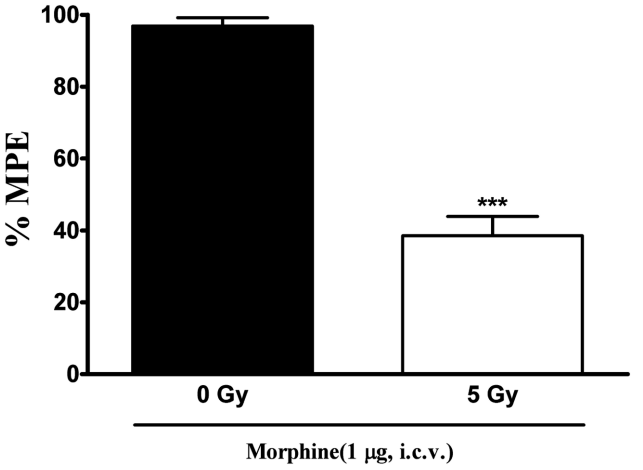

Fig. 8. The effect of whole body irradiation (WBI) stress on the morphine (i.c.v.)-induced antinociception in the hot water tail-immersion test. Mice were whole body irradiated with 5 Gy of γ-ray, and allowed to rest for 120 min. Morphine (1 μg/5 μl) was injected into the mouse third ventricle 20 min before the test. % MPE denotes the percentage of the maximal possible effect. All data are represented as the means ± S.E.M. (n = 8-10). ***p < 0.001 vs. sham Morphine(1 μg/5 μl).

and β-endorphin were injected 20 min for morphine and 30 min for β-endorphin, respectively, prior to the test. The i.c.v. administration of morphine (1 μg) and β-endorphin (0.2 or 1 μg) induced pronounced antinociceptive effects in sham-treated mice. The effect of morphine was significantly attenuated by WBI (Fig. 8.). Like the writhing test, the effect of β-endorphin was potentiated by WBI exposure (Fig. 7.).

Discussion

The mechanisms of radiation-induced oxidative stress are complicated, multi-factorial, and still incompletely defined. It is known that radiation can influence on human cells which may involve every major organ system. Ionizing radiation such as gamma rays is released by unstable atoms that have an excess of energy. WBI to gamma rays, which result in oxidative stress, can penetrate through the body. Ionizing radiation can interact directly with cellular macromolecules such as DNA, mRNA and proteins to break their covalent bonds. Ionizing radiation can also indirectly interact with cells by causing hydrolysis reaction of cellular water resulting in hydrogen molecules and hydroxyl (free radical) molecules (Katanyutanon *et al*., 2008).

The antinociceptive potency of opioids may be influenced by various stress. In most instances, the exposure of experimental animals to a variety of stressful manipulations initiates increases in baseline nociceptive thresholds. The findings of the present study also demonstrate that exposure to WBI stress at a dose of 5 Gy produces an antinociception in the acetic acid-induced writhing and the hot water tail-immersion tests.

In the present study, morphine (i.c.v.)-induced antinociceptive effect was also attenuated in the both tests by oxidative stress. Interestingly, its effect was significant in the hot water tail immersion test, but not in the writhing test. 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 the our and other results, the acetic acid-induced writhing test is more sensitive pain model than the thermal tail-flick test, and thus needs less dose of morphine in producing same level of the antinociceptive effect. As an alternative explanation to this result, it could be due to the modality difference between thermal and chemical noxious stimuli. However, we cannot exclude the other possibility.

Contrary to the antinociceptive effect of morphine by WBI stress, β-endorphin-induced antinociception was not attenuated, but increased (or potentiated) by WBI stress. In addition, this finding extended in part our earlier work by demonstrating that depletion of glutathione in the brain resulted in an attenuation of the acute antinociceptive effect of morphine, but not that of β-endorphin, in mice (Chung *et al*., 2003). Although the data are very interesting, we can presently not explain the exact mechanism responsible for the potentiation of β-endorphin antinociception by WBI stress. It has been known that morphine and β-endorphin given i.c.v. binds μ- and ε-opioid receptor, respectively, and produce their antinociceptive effects through a different action mechanism (Jung *et al*., 1989; Suh *et al.*, 1989; Chung *et al*., 1997, 2003; Chung and Suh, 2001; Tseng, 2001; Seo *et al*., 2008). In addition, Raffa *et al*. (1988) 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 receptors. Since the present study was focused on the examining the effects of whole-body ionizing γ irradiation on the antinociceptive effects induced by morphine and β-endorphin as assessed by the thermal tail withdraw and chemical writhing tests, further studies are necessary to understand the exact antinociceptive mechanisms involved in WBI stress.

In summary, the antinociceptive effect was produced time-dependently and reached its maximum at 120 min after WBI with γ-ray (5 Gy). In addition, the antinociceptive effect produced by β-endorphin (i.c.v.) was potentiated, whereas that of morphine was attenuated by the WBI stress. These results demonstrate a differential sensitivity of μ- and ε-opioid receptors to WBI stress, in addition, support the hypothesis that morphine and β-endorphin administered supraspinally produce antinociception by different neuronal mechanisms.

Acknowledgements

This work was financially supported by the Research Fund from Gangneung-Wonju National University (2007).

References

Ben-Bassat J, Peretz E, Sulman FG. Analgesimetry and ranking of analgesic drugs by the receptacle method. Arch Int Pharmacodyn Ther. 1959;122:434-47.

Chung KM, Suh, HW. Pretreatment with cholera or pertussis toxin differentially modulates morphine- and beta-endorphin-induced antinociception in the mouse formalin test. Neuropeptides 2001;35(5-6): 197-203,.

Chung KM, Jung YK, Jeong HY, Kim KN. Differential involvement of glutathione in morphine and β-endorphin induced antinociception in male ICR mice. Int J Oral Biol. 2003;28(2):33-7.

Chung KM, Park KP, Kim JS, Lee JH. Effects of morphine and β-endorphin administered intracerebroventricularly on the release of neurotransmitters in medullary dorsal horn. J Dent Coll. S.N.U.1997; 21(1):132-52.

Haley TJ, McCormick WG. Pharmacological effect of produced by intracerebral injections of drugs in the conscious mouse. Br J Pharmacol. 1957;12:12-5.

Jung JS, Song DK, Suh HW, Kim YH. Effects of intraventricular injection of morphine and beta-endorphin on serotonin release from the spinal cord in rats. Pharmacol Biochem Behav 1994;49(4):1037-424.

Katanyutanon S, Wu R, Wang P. The effect of whole-body radiation on blood levels of gastrointestinal peptides in the rat. Int J Clin Exp Med.2008;1:332-7.

Koster R, Anderson M, Beer EJ. Acetic acid for analgesic screening. Federal Proceeding 18: 412.

Mahajan HS, Mishra TK, Chakrabarty K, Chakrabarty AS. Immobilisation stress induced analgesia in diabetic rats. Indian J Physiol Pharmacol. 1993;41:275-9.

Mickley GA, Stevens KE, White GA, Gibbs GL. Changes in morphine self-administration after exposure to ionizing radiation: evidence for the involvement of endorphins. Life Sci. 1984;33:711-8.

Niels WH, Roger JB. Manual on radiation dosimetry. Marcel Dekker Inc., New York, 1970.

Raffa RB, Mathiasen JR, Brown DQ. μ-, but not δ-, opioid receptor-mediated antinociception in mice is attenuated by γ-irradiation. Brain Res. 1988;447(2):393-7.

Seo YJ, Kwon MS, Choi HW, Jang JE, Lee JK, Jung JS, Park SH, Suh HW. The differential effect of morphine and beta-endorphin administered intracerebroventricularly on pERK and pCaMK-II expression induced by various nociceptive stimuli in mice brains. Neuropeptides 2008;42(3):319-30.

Suh HH, Fujimoto JM, Tseng LF. Differential mechanisms mediating beta-endorphin- and morphine-induced analgesia in mice. Eur J Pharmacol. 1989;168(1):61-70. .

Suh HW, Chung KM, Song DK, Huh SO, Kim YH. Involvement of supraspinal glutamate and GABA receptors in cold water swimming stress-induced antinociception in mice. Biogenic Amines 1999;15:243-50.

Terman GW, Shavit Y, Lewis JW, Cannon JT, Liebeskind JC. Intrinsic mechanisms of pain inhibition: activation by stress. Science 1984;226:1270-2.

Teskey CC, Kavaliers M. Ionizing radiation induces opioid-mediated analgesia in male mice. Life Sci. 1984;35:1547-52.

Tseng LF. Evidence for ε-opioid receptor-mediated β-endorphin-induced analgesia. TINS 2001:2(12):623-30.

Watkins LR, Mayer DJ. Organization of endogenous opiate and nonopiate pain control systems. Science 1982;216:1185-92.

Yang GY, Park YH, Lee MK, Kim SK, Ahn DK. Compression of the trigeminal ganglion enhances nociceptive behavior produced by formalin in the orofacial area of rats. Int J Oral Biol. 2008;33(4):155-62.