

Endogenous Opioids and Analgesic Effects of Ionizing Radiation in Rats

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Received August 25, 2003

Accepted June 17, 2004

Abstract

Kereškényiová E., B. Šmajda: *Endogenous Opioids and Analgesic Effects of Ionizing Radiation in Rats*. Acta Vet. Brno 2004, 73: 195-199.

Some stressors can cause a temporary decrease of sensitivity to pain (stress-induced analgesia, SIA) in mammals. Ionizing radiation belongs to non-specific stressors, too. The aim of this study was to analyze the effects of ionizing radiation on pain sensitivity in laboratory rats and to establish whether the release of endogenous opioids in post-irradiation period is involved in analgesic effect of radiation.

Two-month-old (310-340 g) intact male Sprague-Dowley rats divided in four groups (n = 10) housed in groups of five were used in the experiments. Rats were held under an LD 12:12 artificial light regimen at temperature of 22 °C and relative air humidity of 60-70%. Food and water were available *ad libitum*. The rats were tested in a hot plate apparatus with a surface temperature of 55 ± 0.5 °C.

It was found that gamma irradiation with a whole body dose of 6 Gy or with a dose of 10 Gy on the head caused significant prolongation of the hind-paw licking latency in the hot plate test. Intraperitoneal administration of naloxone, a blocker of the endogenous opioid μ -receptors in a dose of $8 \text{ mg} \cdot \text{kg}^{-1}$ b.m. 30 min before testing on the hot plate significantly ($p < 0.05$) reversed the post-irradiation analgesia in both irradiation models, while a dose of $4 \text{ mg} \cdot \text{kg}^{-1}$ was ineffective. The results suggest that effects on the CNS may be involved in post-irradiation SIA and that the endogenous opioids probably play an important role in this phenomenon.

Hot plate, ionizing radiation, naloxone, nociception, rat

The discovery of opioid receptors and the subsequent isolation of their endogenous ligands has led to an enormous interest in the physiological significance of these peptides (Malick and Bell 1976). It has been established soon (Morley et al. 1980; Chance et al. 1978), that after physical or psychological stress both central and peripheral levels of endogenous opioids are elevated in laboratory rodents.

Recently, the physiological and behavioural effects of a variety of stressors were further elucidated. In experiments in non-humans, the effects of foot-shock (Ahmed et al. 2000; Foo and Helmstetter 2000), forced swimming (LaBuda et al. 2000; Takahashi et al. 2000), restraint/immobilization (Aloisi et al. 1999) and noxious heat (Hawranko and Smith 1999) on nociception were analysed. The increase of the nociceptive threshold in general is termed stress-induced analgesia (SIA). Although several neurotransmitters in the brain and spinal cord have been implicated, the full details of the neuronal pathways involved have not yet been elucidated.

The aim of our experiments was to study the analgesic effects of ionizing radiation, which could be considered as an unspecific stressor. Further, we wanted to establish, whether the release of endogenous opioids in post-irradiation period is involved in analgesic effect of radiation. To quantify the degree of analgesia, we used one of standard tests of acute pain, the hot plate test (Espejo and Diego 1993; Espejo and Gil 1998).

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Materials and Methods

Animals

Experimentally naive 60-day-old (310-340 g) male Sprague-Dawley rats were housed in groups of five. They were reared under an artificial light regimen of LD (light : dark) of 12:12 hours at temperature of 22 °C and relative air humidity of 60-70%. Food and water were available *ad libitum*.

Experimental groups

Group 1 (n = 10): controls were given saline (CON), Group 2 (n = 10): were irradiated with saline (IRR), Group 3 (n = 10): controls with naloxone (NAL), Group 4 (n = 10): were irradiated with naloxone (IRR+NAL).

Apparatus

We used a modified version of the hot plate apparatus after Espejo and Diego (1993) consisting of a glass chamber with removable cover. The bottom of the chamber was heated to 55 ± 0.5 °C by a 150 W infrared lamp placed under the plate.

Procedure

Animals were irradiated (or sham-irradiated) individually in irradiation boxes with gamma-rays from a ^{60}Co source (Chisostat apparatus, Chirana, Prague, Czech Republic).

Thirty minutes after the irradiation the animals were injected with naloxone (Sigma GmbH, Germany), a specific blocker of opioid μ -receptors or saline (Infusia a.s, Czech republic). Naloxone was applied in a solution containing 1 mg naloxone in 2 ml of saline. Sixty minutes after irradiation the rats were placed individually onto the hot plate and the latency time up to the first licking of one of the hind-paws was recorded.

Experiment 1

The animals were irradiated (or sham-irradiated) by a whole-body dose of 6 Gy and received 4 mg·kg⁻¹ (b.m.) of naloxone or equivalent volume of saline.

Experiment 2

The animals were irradiated (or sham-irradiated) by a whole-body dose of 6 Gy and received 8 mg·kg⁻¹ (b.m.) of naloxone or equivalent volume of saline.

Experiment 3

The animals were irradiated (or sham-irradiated) with a dose of 10 Gy of gamma-rays on the head only (the rest of the body was shielded by a layer of lead) and received 8 mg·kg⁻¹ b.m. of naloxone or equivalent volume of saline.

Statistical analysis

The group mean values were compared using the non-parametric Mann-Whitney test.

The experiments were conducted according to the principles provided in the Act No. 115/1995 § 24 of Slovak republic for the Care and Use of Laboratory Animals.

Results

Experiment 1

Results are shown in Fig. 1. The irradiation caused a significant ($p < 0.01$) increase in hind-paw licking response latency in the hot plate test. Administration of 4 mg·kg⁻¹ b.w naloxone intra-peritoneally had no effect on paw-licking latency in irradiated animals. The administration of naloxone to non-irradiated animals did not influence the followed parameter.

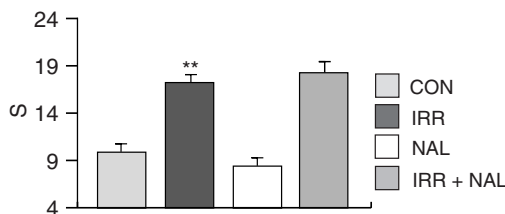


Fig. 1. The effect of whole body irradiation with gamma rays (6 Gy) and of naloxone (4 mg·kg⁻¹) on response latencies in hot plate test in rats. Each bar shows mean \pm SEM. CON - non-irradiated controls with saline, IRR - irradiated with saline, NAL - non-irradiated with naloxone, IRR+NAL - irradiated with naloxone
** $p < 0.01$ (IRR vs CON)

Experiment 2

Increasing the dose of naloxone from 4 to 8 mg.kg⁻¹ b.m. resulted here in statistically significant ($p < 0.05$) reverse of analgetic effect of radiation in hot plate test (Fig. 2). The administration of naloxone to non-irradiated animals did not influence the followed parameter.

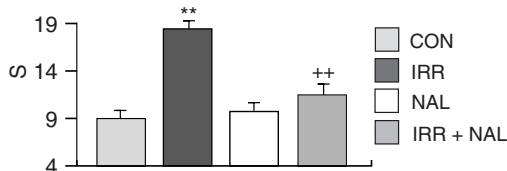


Fig. 2. The effect of whole body irradiation with gamma rays (6 Gy) and of naloxone (8 mg.kg⁻¹) on thermal response latencies in hot plate test in rats. Each bar shows mean \pm SEM. Other details as in Fig. 1.

** $p < 0.01$ (IRR vs CON), ++ $p < 0.01$ (IRR+NAL vs IRR)

Experiment 3

As shown in Fig. 3, irradiation on the head only revealed also a statistically significant ($p < 0.05$) analgetic effect. Naloxone in a dose of 8 mg.kg⁻¹ significantly reversed the prolonged response latencies in animals irradiated cranially. The administration of naloxone to non-irradiated animals did not influence the followed parameter.

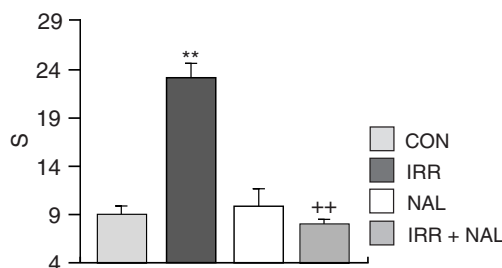


Fig. 3. The effect of cranial irradiation (10 Gy) and of naloxone (8 mg.kg⁻¹) on response latencies in hot plate test. Each bar shows mean \pm SEM. Other details as in Fig. 1.

** $p < 0.01$ (IRR vs CON), ++ $p < 0.01$ (IRR+NAL vs IRR)

Discussion

It has been well demonstrated in recent years, that a range of various stressors causes analgetic response in experimental animals (review see by Vaccarino and Kastin (1999; 2000)). As revealed by application of opioid antagonists, opioid as well as non-opioid mechanisms can be involved in SIA. The opioid receptor subtypes mediating SIA have been found different among different stressors. For example, analgesia induced by forced walking in mice could be blocked by an ϵ -opioid antagonist, but not by the μ - and partly δ - and κ -opioid antagonist naloxone (Nakagawasa et al. 1999).

Early after the discovery of endogenous opioids in the 1970s the effects of ionizing radiation on the opioid mechanism were studied. Teitelbaum et al. (1979) observed that exposure to radiation led to behavioural and physiological responses that resemble those observed after administration of the exogenous opiate, morphine. Beta-endorphin levels were found to be elevated in irradiated mice (Mickley et al. 1983a; Mickley et al.

1983b). Teskey and Kavaliers (1984) showed, that exposure of CF-1 mice to doses of ionizing radiation as low as 2.5 Gy caused an increase in their nociceptive thresholds in the hot-plate test, indicating analgesia. These delayed thermal responses observed following radiation treatment could be blocked and reversed by naloxone.

Our results after applying whole-body irradiation (Experiment 1 and 2) are in good agreement with these findings. However, in contrast to results in mice, relatively high doses of naloxone (8 mg·kg⁻¹ vs 1 mg·kg⁻¹) were needed to elicit the reversal of radiation-induced SIA in rats. This difference in sensitivity to naloxone could be due to inter-species and inter-strain differences in the ratio of μ - to δ -opioid receptors (Castellano and Oliveiro 1975).

It is still not clear, if the stress-effect and SIA caused by ionizing radiation are mediated by overall effects of radiation on radiosensitive tissues in the body, or by its direct effect on the nervous tissue in CNS. Analgetic effects of radiation were reported also by repeated irradiation of mice with a daily dose as low as 0.5 Gy during 6 days (Miyachi 1997). Our results with irradiation on the head only, where SIA sensitive to naloxone was observed, suggest a direct influence of radiation on central opioid mechanisms.

Our results suggest that the effects on CNS may be involved in SIA after irradiation and that the endogenous opioids play probably an important role in post-irradiation analgesia in rats.

Endogénne opioidy a analgetické účinky ionizujúceho žiarenia u potkanov

Niektoré stresové stimuly môžu u cicavcov vyvolať dočasné zníženie citlivosti na bolesť (tzv. stresom indukovaná analgézia, SIA). Medzi nešpecifické stresory patrí aj ionizujúce žiarenie. Cieľom tejto práce bolo analyzovať účinky ionizujúceho žiarenia na citlivosť voči bolesti u laboratórnych potkanov a zistiť, či uvoľnenie endogénnych opiátov po ožiarení zohráva úlohu pri vzniku postiradiačnej analgézie.

Použili sme 60 dní staré (310-340 g) intaktné samce kmeňa Sprague-Dowley, ktoré boli chované v kliečkach po 5. Zvieratá boli chované pri umelom svetelnom režime LD 12:12 pri teplote 22 °C a relatívnej vlhkosti 60-70 %. Potrava a voda boli prístupné ad libitum. Potkany boli testované v teste na horúcej platni pri teplote povrchu platne 55 ± 0.5 °C. Každá pokusná skupina pozostávala z 10 zvierat.

Zistili sme, že gama žiarenie v dávke 6 Gy aplikované celotelovo alebo v dávke 10 Gy aplikované kraniálne spôsobuje u potkanov signifikantné ($p < 0.05$) predĺženie latencie olizovania zadných končatín v teste na horúcej platni, poukazujúcu na vznik analgézie. Intraperitoneálne podanie blokátora μ -receptorov opioidov, naloxónu, v dávke 8 mg·kg⁻¹ hmotnosti i.p. 30 minút pred testom na horúcej platni dokázalo štatisticky signifikantne zvrátiť postiradiačnú analgéziu u oboch spôsobov ožiarenia, kým dávka 4 mg·kg⁻¹ bola neúčinná.

Dosiahnuté výsledky poukazujú nato, že za vznik SIA po ožiarení môžu byť zodpovedné účinky žiarenia na úrovni CNS a že endogénne opiáty hrajú pravdepodobne dôležitú úlohu pri vzniku postiradiačnej analgézie u potkanov.

Acknowledgements

The project was supported by the Grant Agency of Slovak Ministry of Education VEGA (grant No. 1/0440/03).

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