IAEA-CN-67/133

Analgesia Induced by Repeated Exposure to Low Dose X-Rays in Mice, and Involvement of the Accessory Olfactory System in Modulation of the Radiation Effects

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ABSTRACT: The effects of low-dose X-rays on mouse nociceptive behavior were examined using a formalin injected test which rated the amount of time the animals spent licking the injected hind-paw. Male ICR White Swiss mice showed a marked suppression of licking behavior after repeated low-dose X-irradiation (5 cGy/day, 6 consecutive days). The most profound effect was observed on the day 30 after irradiation. The decline of licking behavior, however, was not observed at all following olfactory bulbectomy or vomeronasal tract cut. The analgesic effects could be observed in writhing animals administered acetic-acid intraperitoneally. Moreover, analgesia was totally blocked by the administration of *N*-nitro-L-arginine, a nitric oxide synthase inhibitor, to accessory olfactory bulbs prior to the exposure. The present results indicate that the olfactory system plays an important role in modulation of radiation-induced analgesia, and a possible involvement of nitric oxide in the formation of recognition memory subjected to repeated X-rays. Relatively higher doses (5 cGy × 9 days, 5 cGy × 12 days), however, did not induce such effects, namely, the decline of nociceptive response was limited to the animals irradiated with the smaller dose.

INTRODUCTION

It is well known that many noxious, non-noxious and environmental stimuli can induce changes in pain reactivity. Most commonly, a rise in the nociceptive threshold, antinociception, has been described in rodents following the administration of electric foot shock, immobilization or warm and cold water swims. This phenomenon is termed stress-induced analgesia. 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.

X-rays also can be classed as physical stressors. But X-ray stimulus is particular, as X-rays cannot be perceived by the sensory organs of mammals. It is of interest to determine whether or not X-ray stress produces analgesia similar to that caused by perceivable stimuli such as electric foot shock.

Stress-induced analgesia is thought to be mediated by endogenous pain inhibitory systems, involving both opiate and non-opiate mechanisms. Enkephalin, an endogenous opiate-like peptide, is a neurotransmitter known to have various effects on the brain, including decrease of pain, alteration of response to stress, and modification of the neuroendocrine activity. We reported that brain Met-enkephalin in rodents rapidly decreased after 10 to 20 cGy X-irradiation [1, 2]. The most profound effect of radiation was observed in the hypothalamus. The secretion presumably provide the organism with means of adaptating to stressful environments such as X-irradiation. But no report has yet dealt with changes in nociceptive behavior induced by low-dose radiation of less than 50 cGy. We have therefore examined the effect of low-dose X-rays on the behavioral changes induced by pain, using an inflammatory agents such as formalin or acetic acid.

We recently reported that a brief exposure to X-rays served as an arousal stimulus in mice [3]. Exposure to X-rays (4 cGy) induced an immediate change in EEG patterns from low-frequency and high-amplitude (sleep) to high-frequency and low-amplitude (arousal). In anosmia mice by olfactory bulbectomy, no arousal response could be

observed. These results indicate that the olfactory bulbs play an important role in the immediate detection of X-rays.

To confirm the involvement of the olfactory system in the radiation effects on the nociceptive behavior, the anosmia mice following bulbectomy or vomeronasal tract cut were also examined.

RESULTS

Changes in licking behavior after repeated low-dose X-irradiation are shown in Table 1. After 5 cGy × 3 days irradiation, the time intervals which animals spent licking the injected paw tended to be slightly lower than in the sham-control. The depressive effect became remarkable when the radiation dose was elevated to 5 cGy × 6 days. The significant decrease was already noticed on the day 10, and the most profound effect was observed on the day 30. It reached a value of about one-fourth of the control.

Increasing the radiation dose further 5 cGy × 9 days or × 12 days, however, resulted in the complete disappearance of the effects, namely, the mice irradiated with the higher doses showed no differences from the sham control. These results suggest that the decrease in nociceptive effects is limited to animals irradiated with the smaller dose.

As shown in Table 2, the number of writhing response decreased significantly on the day 30 after 5 cGy × 6 days exposure, reaching a value that was 25 % of the control. Furthermore, increasing the radiation dose caused the complete disappearance of the effects. The suppression of writhings corresponded to that of licking behavior after 5 cGy × 6 days.

As shown Table 3, the time intervals of paw-licking in sham-control of all groups decreased markedly, namely, analgesia was clearly induced by 5 cGy for 6 days exposure. The licking behavior, however, increased significantly and reached to the level of non-irradiated group following OB or VNZ treatment that caused a dysfunction in the main and accessory olfactory system. Interestingly, the licking response of the animals receiving VN transaction also increased significantly. There was no difference, however, between the control and Z treatment, which could be disruption of only the main olfactory system. The present results suggest that the integrity of the accessory system is far more important than the main olfactory system for the expression of radiation-induced analgesia.

Table 4 showed that the licking behavior in mice injected with the NO synthase inhibitor intraperitoneally increased significantly compared to saline-treatment groups, and reached the level of the non-irradiated mice. The effect upon writhing response was the same. The licking response increased markedly in mice given a local infusion of nitro-arginine, compared with those given saline. The results indicate the complete disappearance of the radiation-induced analgesia.

Table 1. Depressive effects of mouse's licking behavior induced by low-dose X-irradiation.

	Day 10	Day 20	Day 30
Sham-control groups	196.5 ± 21.8 (15)	191.5 ± 20.6 (15)	188.2 ± 18.4 (15)
Split exposed groups			
5 cGy × 3 days	155.6 ± 25.3 (15)	159.6 ± 18.3 (15)	196.6 ± 16.3 (15)
5 cGy × 6 days	95.2 ± 14.6 (15) **	92.6 ± 15.0 (15) **	47.1 ± 17.3 (15) ***
5 cGy × 9 days	163.9 ± 19.5 (15)	174.3 ± 20.1 (15)	190.4 ± 20.5 (15)
5 cGy × 12 days	162.4 ± 18.5 (10)	172.3 ± 24.1 (10)	200.3 ± 17.1 (10)
Single exposed groups			
30 cGy	196.4 ± 25.8 (10)	175.9 ± 18.7 (10)	220.3 ± 22.1 (10)

Licking behavior was measured on days 5, 10, 20 and 30 after low-dose X-irradiation.
Data represent as an accumulated interval of licking after injection of formalin (sec/0-30 min).
Significant (*** $p<0.01$, ** $p<0.02$) when compared to sham-control. Values are mean ± S. E.
Figures in parentheses are numbers of mice used.

Table 2. Depressive effects of mouse's writhing response induced by low-dose X-irradiation.

	Day15	Day 30
Sham-control groups	45.0 ± 1.3 (15)	44.2 ± 1.8 (15)
Split exposed groups		
5 cGy × 3 days	46.1 ± 2.0 (15)	26.4 ± 2.4 (15) **
5 cGy × 6 days	40.3 ± 2.9 (15)	10.3 ± 1.9 (15) ***
5 cGy × 9 days	48.4 ± 2.4 (15)	38.4 ± 2.8 (15)
5 cGy × 12 days	39.9 ± 2.5 (10)	41.9 ± 2.5 (10)

Wrthing response was measured on days 15 and 30 after low-dose X-irradiation.
Significant (*** $p<0.01$, ** $p<0.02$) when compared to sham-control.
Values are mean ± S. E. Figures in parentheses are numbers of mice used.

Table 3. Disappearance of radiation-induced analgesia by olfactory bulbectomy or vomeronasal tract cut

Experimental condition	Licking response	Writhing response
Non-irradiated group	172.3 ± 14.9 (10)	48.5 ± 1.8 (10)
Irradiated groups		
OB sham	53.2 ± 14.6 (10)	11.2 ± 0.8 (10)
OB	184.4 ± 19.6 (10) ***	42.6 ± 2.3 (10) ***
VN sham	48.4 ± 13.8 (10)	9.8 ± 0.9 (10)
VN	159.3 ± 22.8 (10) ***	43.7 ± 2.0 (10) ***
Z sham	63.4 ± 18.1 (10)	13.5 ± 1.1 (10)
Z	84.9 ± 24.6 (10)	22.6 ± 2.7 (10)
VNZ sham	57.6 ± 17.5 (10)	13.5 ± 2.2 (10)
VNZ	162.9 ± 20.5 (10) ***	41.6 ± 2.4 (10) ***

The test was performed on day 30 which was observed the profound depressive effects of nociceptive response after 5 cGy for 6 days exposure. Significant (*** $p<0.01$) when compared to sham-control. Values are mean ± S. E. Figures in parentheses are numbers of mice used.
OB=Olfactory bulbectomy, VN=Vomeronasal tract cut, Z=$ZnSO_4$ nasal perfusion, VNZ=$ZnSO_4$ + Vomeronasal tract cut.

Table 4. Disappearance of radiation-induced analgesia by *N*-nitro-L-arginine given prior to the exposure

Experimental condition	Licking response	Writhing response
Non-irradiated group	174.6 ± 18.9 (10)	47.3 ± 2.4 (10)
Irradiated groups		
i. p. saline (sham-control)	53.4 ± 18.1 (10)	11.3 ± 0.7 (10)
i. p. nitro-arginine	144.8 ± 17.6 (10) ***	44.5 ± 1.8 (10) ***
l. i. saline (sham-control)	58.7 ± 17.9 (10)	14.6 ± 0.9 (10)
l. i. nitro-arginine	163.7 ± 20.5 (10) ***	48.9 ± 2.7 (10) ***
l. i. bicuculline	97.8 ± 19.4 (10)	28.6 ± 3.4 (10)

The test was performed on day 30 which was observed the profound depressive effects of licking behavior after 5 cGy for 6 days exposure. Significant (*** $p<0.01$) when compared to sham-control. Values are mean ± S. E. Figures in parentheses are numbers of mice used.
i.p=intraperitoneal injection, l.i=local infusion.

DISCUSSION

It is well known that exposure to stressful stimuli perceivable by sensory organs of mammals such as electric foot shock can produce analgesia. X-rays, a unperceivable agent, also can induce apparent analgesia. When the mice were deprived of their olfactory sense by OB VN or VNZ, the decline of licking response after the exposure was not observed at all. There was no difference, however, between the control and Z groups in which only the main olfactory system was disrupted. The present results suggest that the integrity of the accessory system is far more important than the main olfactory system for the expression of radiation-induced analgesia.

The other interesting finding reported here is that the effect was only observed in the lower dose range (5 cGy × 6 days), and not in cases of relatively higher doses (5 cGy × 9 days or × 12 days).

Stress-induced analgesia typically lasts only a few hours. The analgesia shown here, however, continued for up to 4 weeks after the exposure. We think that though little is known about the mechanisms, a long time-interval after irradiation might be essential to induce the low-dose radiation effects, in contrast to common stressors such as electric foot shock.

NO is a simple gas which possesses a radical structure under atmospheric conditions. Accumulating evidence suggests that NO simultaneously serves as a messenger for neurons, much like a neurotransmitter, in brain. A central focus in neurobiology is the growing interest in the search for the mechanisms underlying the changes in synaptic plasticity characteristic of learning and memory, as well as other neural phenomena. The results presented here showed that the radiation-induced analgesia was totally blocked by administering NO synthase inhibitor to the AOB, prior to the exposure. We think that the recognition memory subjected to repeated X-rays might be formed by NO in the accessory olfactory system.

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