Rapid Decrease in Brain Enkephalin Content after Low-dose Whole-body X-Irradiation of the Rat

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Methionine-enkephalin (ME) contents in the hypothalamus and other rat brain structures were measured immediately after 10 or 20 cGy whole-body X-irradiation. The ME contents of homogenates of the striatum, hypothalamus, midbrain-thalamus, hindbrain and pituitary were assayed radioimmunologically with 125I. The contents of all the structures, except the pituitary, decreased significantly after 20 cGy irradiation. The reduction in the hypothalamus was transient, ME content gradually recovering with time. These results suggest that the central nervous system of mammals is one of the most radiosensitive organs as judged by changes in stress-induced mediators such as ME.

INTRODUCTION

The central nervous system is a complex network of numerous neurons and transmission of information within it is mediated by neurotransmitters. Enkephalin, an endogenous opiate-like peptide, is a neurotransmitter that has been identified as a mixture of two pentapeptides; methionine-enkephalin (ME) and leucine-enkephalin1). It is reported to have a various effects on the brain, including decrease in pain2), alteration of the response to stress3) and modification of the neuroendocrine activity4). There also is growing evidence that opioid peptides act in the regulation of the immune system5). Little is known about the intrinsic, physiological functions of opioid peptides, but a possibility exists that they may function in physiological events in adaptive changes to stress.

Radiation also can be classed as a physical stress agent. Mickley et al.6) reported that the content of /3-endorphin, an opioid peptide, significantly decreased after whole-body 15 Gy 60Co y-irradiation. But no report has dealt with changes in the opioid peptide content induced by low-dose radiation of less than 20 cGy. We examined the effect of 10 or 20 cGy X-ray on the ME
contents of rat brains. Results clearly show an immediate and rapid decrease in ME content in the brain after 20 cGy whole-body X-irradiation.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (7 weeks old, 220–250 g) were obtained from Charles River Japan Co. Ltd. All animals were kept under controlled conditions (temperature: 22 ± 1°C; light-dark schedule, light given between 7:00 and 19:00) and were acclimated to laboratory conditions for 2 weeks before use in the experiments. Food and water were given ad libitum.

Radiation exposure. The rats were placed in clear plastic containers (188×43×85 mm) approximately 5 min before irradiation or sham irradiation. They were then given whole-body X-ray exposure of 10 or 20 cGy, with an X-ray machine (TOSHIBA, KXC-18) at the dose rate of 20 cGy/min (165 kVp, 20 mA).

Removal of brain structures. Immediately after irradiation or sham-irradiation, the rats were decapitated and their heads removed. The brain was dissected on an ice-cold plate into the striatum, hypothalamus, midbrain + thalamus, hindbrain (medulla oblongata + pons) and pituitary according to the method of Glowinski and Iversen. The treatments were performed 9 a.m. to noon to avoid the influence of circadian rhythm.

Radioimmunoassay of ME. The brain samples were homogenized in acidified ethanol and centrifuged. The resulting supernatant was used for the ME radioimmunoassay, details of which are reported elsewhere. Briefly, ME contents of the supernatants were measured with 125I labeled ME (Du Pont Inc.) and antibodies to authentic ME. The antibodies crossreacted less than 0.1% with leucine enkephalin. No crossreactivity was found with α-, β- or γ-endorphin, adrenocorticotropic hormone, somatostatin, thyrotropin-releasing hormone or luteinizing hormone-releasing hormone.

RESULTS

Decreased ME contents of various brain structures after low-dose X-irradiation. ME contents in the striatum, hypothalamus, midbrain + thalamus and hindbrain immediately after 10 or 20 cGy X-irradiation are shown in Fig. 1. After the 10 cGy irradiation these contents tended to be slightly lower than in the sham-irradiated controls, but the difference was not statistically significant. This tendency became more pronounced when the dose was raised to 20 cGy. ME contents after 20 cGy irradiation of all the brain structures shown in Fig. 1 decreased significantly (P < 0.01), in particular in the hypothalamus, and reached a value one-fifth that of the control. In the pituitary, no significant decrease was found. These results suggest that low doses of ionizing radiation (less than 20 cGy) induce a significant decrease in the ME contents of rat brain structures, except the pituitary.

Recovery of the ME content of the hypothalamus with time after 20 cGy whole-body irradiation. The ME content of the hypothalamus was measured at various times up to 4 hours
Fig. 1. Effects of low-dose ionizing radiation on ME contents of different brain regions. Values are expressed as percentages of the ME contents in sham-irradiated controls. Vertical bars show the standard error of the mean (S.E.M.) for six to nine rats. Significant differences (p < 0.01) between the control and irradiated groups are shown by an asterisk (*). ME content (ng/mg protein + S.E.M.) for sham-irradiated rats: striatum, 7.8 ± 0.4; hypothalamus, 5.3 ± 0.3; midbrain, 3.8 ± 0.3; hindbrain, 4.3 ± 0.4; pituitary, 0.9 ± 0.1.

Fig. 2. Rapid reduction and gradual recovery of ME contents in rat hypothalamus after 20 cGy whole-body exposure. Significant differences (P < 0.01) between the controls and irradiated groups are shown by an asterisk (*). Figures in parentheses are the numbers of rats used. Closed circle: ME content in sham-irradiated control rat brains.
after irradiation by the method described above (Fig. 2). The decrease in ME in the hypothalamus was transient, rapid recovery taking place with time to 65% of the control value by 4 hours after irradiation.

DISCUSSION

The central nervous system which includes the brain, has been believed to be pathologically or functionally one of the most radioresistant organs. The results presented here are evidence against this widely accepted belief. The brain reacts promptly to radiation insult, even to doses as low as 20 cGy (Figs. 1 and 2).

Interestingly, the greatest effect of radiation was found in the hypothalamus, the pituitary being less affected. A number of studies have suggested the existence of two pathways for the release of endogenous opiates; one via the endocrine system including the pituitary gland, the other through the autonomic nervous system. The hypothalamus is involved in the control of the autonomic nervous system, and is linked vascularly with the anterior lobe of the hypophysis in endocrine system. It also appears to function in the mechanisms that underly moods and motivational states. Although the mechanism that underlies the changes in ME contents has yet to be determined, the marked difference in the decrease in ME contents of the hypothalamus and pituitary suggest the complicated release system (mentioned above) of the endogenous opiate.

There are many reports which suggest that endogenous morphine-like substances (opiates) are released as a reaction to stressful situations. These secretions presumably provide the organism means of adapting to its environment; e.g., providing analgesia during foot shock or increasing the activity for adaptation to a stressful environment.

Low dose irradiation may produce a change in the endogenous opiate content thereby inducing a physiological alteration; one, for example, that is related to some kind of defense system such that the animals would become temporarily conditioned to tolerate a lethal radiation dose. The long-term effect of low dose irradiation on ME contents in the brain is now being investigated.

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REFERENCES


