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Changes of Pineal N-Acetyltransferase Activity in Gamma Irradiated Rats

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Summary

Male Wistar rats were exposed to whole body irradiation with 14.35 Gy gamma rays after the adaptation to light/dark cycle (LD 12:12). Three groups of rats were examined: A) rats irradiated in the night and placed in the 12 h LD cycle again, B) rats irradiated in the day-time and placed in the 12 h LD cycle, and C) rats irradiated in the night and kept in constant darkness. All analyses were carried out in the dark. Radiation enhanced the activity of pineal N-acetyltransferase 3-4 days after exposure in all groups, in the C group significantly on the 4th day. Different light regimens during and after irradiation did not to affect the activity of this key enzyme of melatonin synthesis substantially.

Key words

Ionizing radiation – Rats – Pineal N-acetyltransferase activity

Introduction

Information about the effect of ionizing radiation on the pineal gland is very important not only experimentally but especially in clinical practice. The therapeutic irradiation of cancer patients certainly influences their neuroendocrine functions; the elucidation of these alterations is necessary for successful treatment.

As far as the effects of ionizing radiation on the pineal gland are concerned, only a few morphological studies are available; the data about the biochemical changes are sparse (see Barfuss *et al.* 1969, Ellis *et al.* 1970).

The production of the main pineal indoleamine – melatonin is rhythmical, depends on the alteration of light and darkness and is regulated by sympathetic fibres from the superior cervical ganglion, which influence beta-adrenergic receptors in the pinealocyte membrane. The levels of melatonin – the hormone of darkness – are high during the dark part of the day and low during the light part. This corresponds with the rhythm of the key enzyme of melatonin synthesis – N-acetyltransferase (NAT).

Material and Methods

Male SPF Wistar rats, three months old, were adapted to an artificial light-dark regimen (LD) 12:12 h for six weeks under standard vivarium conditions (temperature 22±2 °C, relative humidity 60-70 %). Cool light (fluorescent lamps Tesla, 40 W) of 150 lux intensity in each cage was automatically switched on at 0700 h. The animals had free access to food (ST pellets, Velaz, Praha) and water. The animals were subjected to whole body irradiation with 14.35 Gy from a ^{60}Co – source (Therapeutic Apparatus Chisostat, exposure rate 0.44 Gy per minute). The rats were irradiated fed; after irradiation and sham-irradiation they fasted till the analysis. Three variants of the experiments were carried out : A) rats irradiated in the dark part of the day between 2300-2400 h and kept in the 12 h LD regimen again, B) rats irradiated in the light part of day, between 1100-1200 h and placed in the 12 h LD cycle again, C) rats irradiated in the dark part of day as in the A group with the exception of the groups examined 6 h after exposure. Those were irradiated between 2100-2200 h. All the animals were placed in constant darkness.

The rats were rapidly decapitated in the darkness 12 h to 4 days after irradiation between 2300-2400 h, 6 h after irradiation between 0300-0400 h. They were sacrificed 24 h after irradiation in the B group between 1100-1200 in the light. A dim red light of less than 1 lux intensity was employed at each manipulation in the dark (irradiation, sham-irradiation, sacrifice). Pineal N-acetyltransferase activity was determined according to Deguchi and Axelrod (1972) in a modification according to Parfitt *et al.* (1975). Each group consisted of 6 to 8 rats. The results were evaluated using the non-paired t-test.

Results

N-acetyltransferase activity was enhanced within 3-4 days after irradiation in all the experimental groups, the increase was significant on day 4 (Fig.1). Neither the different time of the day of exposure to ionizing radiation (night: A, C; day:B) nor the different light-dark regimen after irradiation influenced NAT activity. The pineal weight of irradiated and non-irradiated animals did not differ.

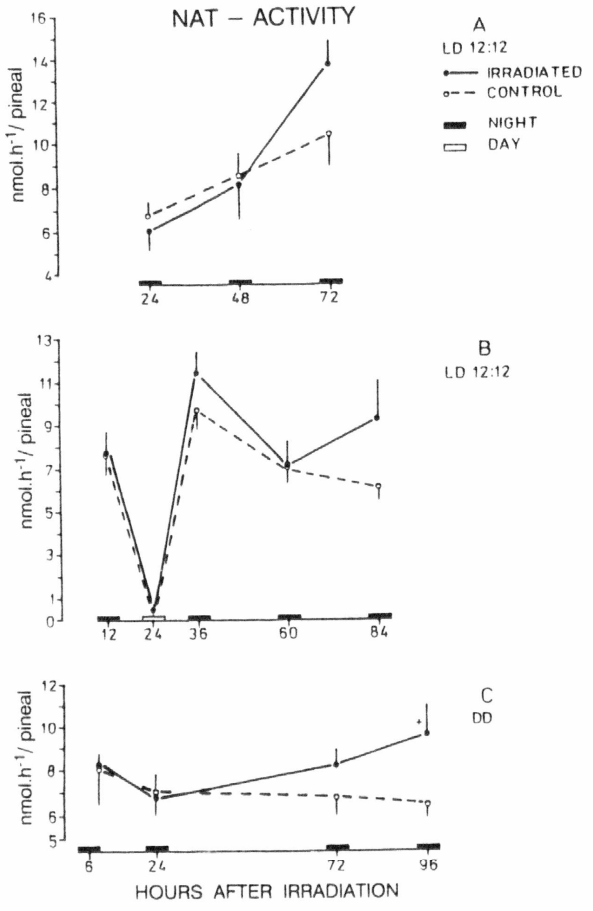

Fig. 1
Pineal N-acetyltransferase activity in irradiated and sham-irradiated rats at various intervals after exposure to a dose 14.35 Gy of gamma-rays. A – rats irradiated in the dark part of day (2300-2400 h), kept after exposure in the LD 12:12 regimen again and examined in the darkness. B – rats irradiated in the light part of day (1100-1200 h), placed after exposure in the LD 12:12 regimen and examined in the darkness except the 24 h interval. C – rats irradiated in the dark part of day as the A group, placed after exposure and examined in constant darkness (DD). Values are given as means ± S.E.M. Diferences between groups were considered significant at $p<0.05$.

Discussion

The data about the effect of ionizing radiation on pineal function are sparse. Barfuss *et al.* (1969) found a decrease in pineal hydroxyindole-O-methyltransferase activity (an enzyme, which catalyzes the transformation of N-acetylserotonin to melatonin, HIOMT) 24 h after irradiation of male rats with 450 R of X-rays. The rats were maintained in natural light, the time of exposure and of analysis is not mentioned. Ellis *et al.* (1970) investigated the dynamic changes in HIOMT activity during 31 days in the rat's pineal after whole body irradiation with the dose 350 R of X-rays. Under the conditions of natural light, the enzyme activity was decreased 1-24 h after irradiation, it was increased from the 4th to the 19th day and in the following intervals it did not differ from the control group. Pineal HIOMT activity was significantly decreased in whole body-irradiated rats with 450 R of X-rays and maintained in constant light before and after irradiation. It was not significantly changed in rats kept in a natural LD regimen before and after exposure or in the constant darkness, as compared to the control animals. Data about the time of day of the exposures were not given. In both these experiments the rats were killed by an overdose of ether anaesthesia.

Bayer *et al.* (1970) investigated the pineal ultrastructure in female rats exposed to an acute dose of 700 R of X-rays after whole body irradiation with the head shielded and after local irradiation of the head with the body shielded. The changes in the pineal gland 24 h, 48 h and 6 days after irradiation showed a mixture of symptoms of activation and degeneration of the pinealocytes. The nerve endings were not markedly changed. Radiation damage appeared earlier and was more striking after local irradiation of the head. Neurosecretory response of the hypothalamus and posterior pituitary to sublethal and lethal irradiation (from 400 to 1000 R) with X-rays or gamma rays was similar in whole body exposure and in partial irradiation of the head (Duchesne *et al.* 1968). Immediately after irradiation, a release of neurosecretion from the cell cytoplasm of the supraoptic and paraventricular nuclei was noted. The histochemical picture was normalized 11 h after irradiation. The authors described an early neurosecretory response to the radiation stressor. A "secondary" neuroendocrine reaction in rats irradiated with 1000 R of X-rays began later – 24 h after irradiation and culminated 4-5 days after exposure, during fully manifested radiation injury (Duchesne *et al.* 1968).

In our experiment, we found an increase of NAT activity in the pineal gland 3 or 4 days after irradiation, corresponding probably to a secondary reaction of the pineal. We did not observe an early response of NAT activity 6 h after exposure or we may have missed its even earlier occurrence. Ellis *et al.* (1970) described a decrease in HIOMT activity one hour after the exposure to 350 R of X-rays. This fact implies that the direct effect of irradiation on pinealocytes would become manifested within 1-2 h after the exposure.

A substantial part of the pineal reaction to the ionizing radiation could be mediated by activation of the sympathetic system. One day after irradiation with 8.5 Gy of X-rays, the level of norepinephrine was reduced by 61 % in the brain and 37 % in the rat heart (Varagic *et al.* 1967). The level of circulating catecholamines was not changed after exposure to sublethal or lethal doses of X-rays (Griffith *et al.* 1961).

In the future we would like to assess NAT activity, serum and pineal melatonin levels after irradiation of the head alone, namely at earlier time intervals.

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Reprint Requests

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