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THE EFFECT OF A SINGLE WHOLE-BODY IRRADIATION WITH A LETHAL DOSE ON CATECHOLAMINE LEVELS IN THE PINEAL GLANDS OF RATS

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Male Wistar rats adapted to light-dark cycle (LD) 12:12 h were exposed in the darkness to a lethal single dose of 14.35 Gy gamma-rays on the whole body. Irradiation, sham-irradiation and decapitation 30, 60, 120 min after the exposure, were performed between 2000 h and 0100 h in the darkness. The changes in the concentrations of catecholamines (CA) were determined by the radioenzymatic method in the pineal gland of rats. Results were evaluated in comparison with those of the sham-irradiated (30, 60, 120 min) control groups of rats. From the results it follows that the concentration of dopamine (DA) and norepinephrine (NE) in the rat pineal gland significantly decreased 30 and 120 minutes after irradiation ($p < 0.01$). At min 60 of the postirradiation period it was possible to observe a tendency to recovery of the levels of DA and NE to control values. Epinephrine (EPI) exhibited a significant ($p < 0.05$) decrease at min 60 and 120 of postirradiation (by 61.8% and 62.8%, respectively).

Key words: *pineal gland, catecholamines, ionizing radiation, catecholamines, rats.*

INTRODUCTION

Knowledge about the effect of ionizing radiation on the pineal gland is very important not only experimentally but especially in clinical practise. The therapeutic irradiation of cancer patients to the head certainly influences their neuroendocrine functions and the central nervous system.

Ionizing radiation significantly changes the levels and metabolism of catecholamines as well as the amount of α- and β-adrenergic receptors in different irradiated tissues (1, 2). The data on the effect of ionizing radiation on the metabolism of catecholamines in the brain available in literature are various and depend on the kind of radiation, exposure dose and species sensitivity (3, 4).

As there are no data in available literature on the effect of radiation on the pineal catecholamines that play a key role in the regulation of melatonin synthesis, we aimed to assess the changes in the levels of dopamine, norepinephrine and epinephrine in the pineal gland of rats after application of a single whole-body irradiation with gamma-rays with a total lethal dose of 14.35 Gy. The early postirradiation changes in the concentrations of catecholamines 30, 60 and 120 minutes after irradiation were studied.

MATERIAL AND METHODS

Male Wistar rats (n = 30) weighing about 200 g were adapted to a light-dark regimen (12 h light: 12 h darkness) for six weeks under standard vivarium conditions (temperature 22±2°C, relative humidity 60—70%) in November. Cool light (fluorescent lamps Tesla 40 W) of 150 lux intensity per a cage was automatically switched on at 0700 h. The rats had free access to water and food (ST pellets, Velas, Prague). After this adaptation the fed rats were exposed to a single dose of 14.35 Gy of gamma rays on the whole-body from a ^{60}Co source (Therapeutic Apparatus Chisostat), exposure rate 0.38 Gyxmin^{-1} in special perspex boxes. The dose were measured by thermoluminiscent dosimeters (LiF) and evaluated by a victoreen TLD Reader 2800. The controls were sham-irradiated by the same procedure. Irradiation and sacrificing of the rats were carried out in the darkness (between 2000 and 0100).

Determination of catecholamines

After rat decapitation the brain was removed from the cranial cavity and the pineal gland was taken out quickly. After its removal tissue was immediately imersed into liquid nitrogen to prevent degrading changes; it was kept in a frozen state until further processing. Pineal glands were homogenized in the Potter-Elvehjem's glass microhomogenizator in 0.4 mol. l^{-1} $HClO_4$ with addition of reduced glutation (0.05 mol. l^{-1}). The tissue homogenates were centrifuged at 20,000 g. min^{-1} at 0°C for 30 minutes. For radioenzymatic determination 50 µl of supernatant were used. Catecholamines (dopamine, norepinephrine, epinephrine) were determined by the radioenzymatic method acording to Johnson *et al.* (4). After isolation of CA from the tissue supernatants their individual radioactive derivatives were separated chromatographically on the Silufol UV plates. The ^{3}H activity of CA derivatives was measured on a scintillating spectrometer Packard-Tri Carb in a ^{3}H channel. The coefficient of method variation calculated from 10 repetitions of one sample is 4.2% for NE; 4.1% for DA and EPI. The pineal CA statistical analysis levels were expressed as pmol CA. mg of tissue^{-1}, in arithemetical means ±S.E.M. and they were analysed statistically by the non-paired t-test.

RESULTS

The pineal epinephrine was present in sham-control rats in much lower concentrations than norepinephrine. The level of dopamine in sham-irradiated rats was relatively balanced at the time intervals (*Fig. 1*). Application of 14.35

Gy of gamma rays significantly reduced concentration of the pineal DA ($p<0.05$) at 30 and 120 min after exposure (by 56.5% and 42.27%, respectively). At 60 min after irradiation a recovery of DA levels to the control values was recorded (*Fig. 1*). Similar changes were found in concentrations of the pineal norepinephrine (*Fig. 2*). At 30 and 120 min a significant decrease in NE levels was observed ($p<0.01$ and $p<0.05$; by 52.3% and 55.2%, respectively), whereas 60 min after exposure NE, like DA, levels did not significantly differ from those in sham-control groups (*Fig. 2*). Irradiation with 14.35 Gy induced a decrease in concentration of epinephrine 60 and 120 min later by 61.87% and 62.6% ($p<0.01$) respectively (*Fig. 3*).

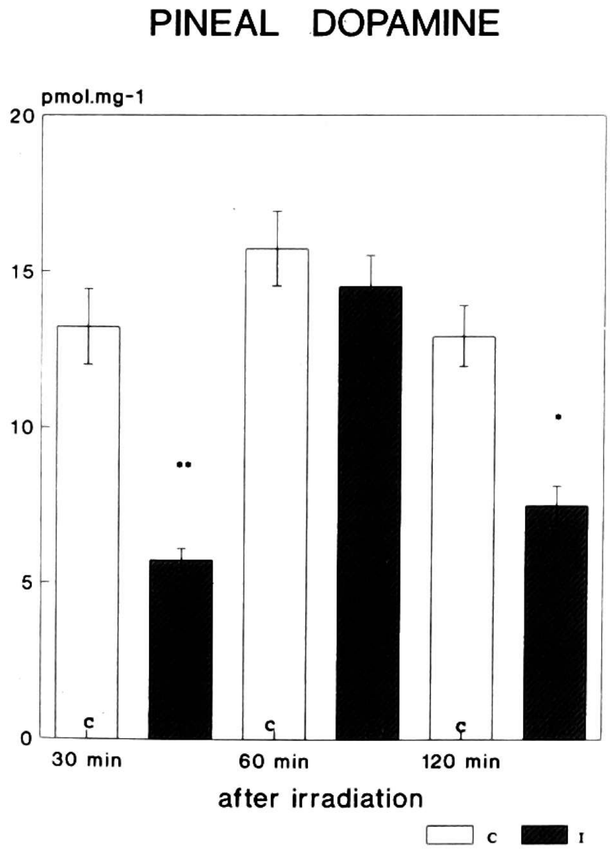

Fig. 1. Postirradiation changes in the pineal dopamine levels of rats 30, 60 and 120 minutes after exposure to a single, whole-body irradiation of rats for 40 minutes from ^{60}Co source with a total dose of 14.35 Gy. Results are expressed in pmol.mg tissue^{-1}, C — the levels of pineal dopamine of "sham" irradiated rats (30, 60, 120 min), I — the levels of pineal DA of rats 30, 60 and 120 minutes after exposure to 14.35 Gy. Significant differences from sham controls * $p<0.05$, ** $p<0.01$.

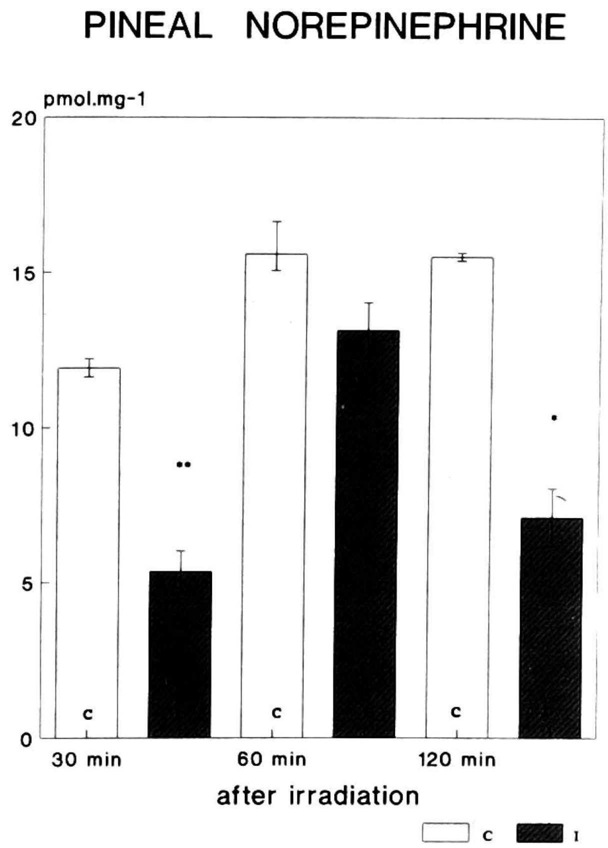

Fig. 2. Postirradiation changes in the pineal norepinephrine of rats 30, 60 and 120 minutes after exposure to a single, whole-body irradiation for 40 minutes from ^{60}Co source with a total dose of 14.35 Gy. Symbols are the same as those in Fig. 1. Significant differences from sham control * $p<0.05$, ** $p<0.01$.

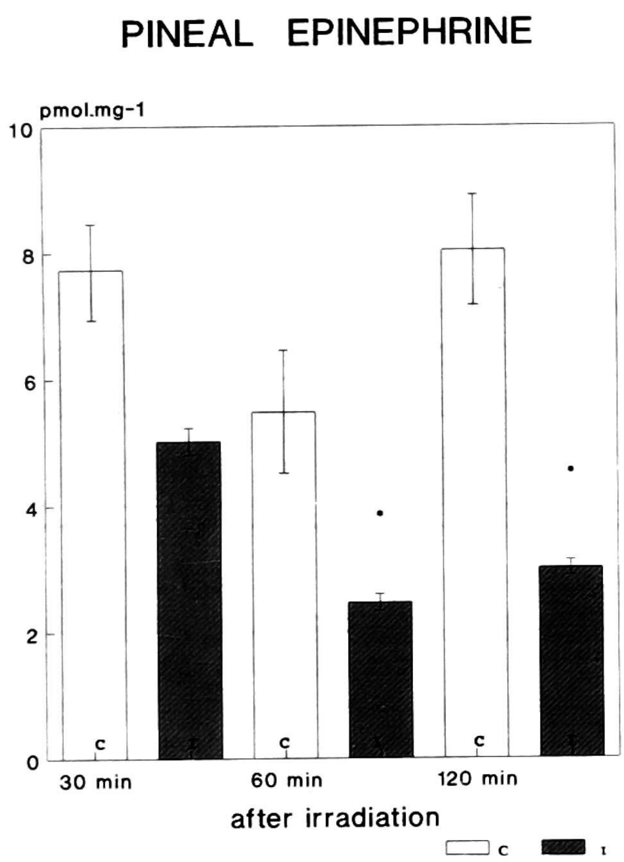

Fig. 3. Postirradiation changes in the pineal epinephrine of rats 30, 60 and 120 minutes after exposure to a single, whole-body irradiation for 40 minutes from ^{60}Co source at a total dose of 14.35 Gy. Symbols are the same as those in *Fig. 1.* * $p<0.05$, ** $p<0.01$.

DISCUSSION

Informations on the effect of gamma irradiation on the pineal gland are scarce in available literature and concern morphological and biochemical changes (5—8).

The pineal norepinephrine is present in the nerve-endings of adrenergic neurons that project from cervical ganglia into the pineal gland. The catecholamines participate in the regulation or modulation of melatonin secretion in the pineal gland. The adrenergic neurons increase their activity in darkness, when the synthesis of pineal norepinephrine is elevated. Norepinephrine acts on α- and β-receptors of pinealocytes and stimulates the activity of serotonin N-acetyltransferase (NAT) a key enzyme of melatonin synthesis in pineal gland (9, 19). After exposure to whole-body ionizing radiation with a dose of 14.35 Gy the increase in the activity of the pineal serotonin N-acetyltransferase was recorded on 3—4 days after gamma-irradiation, whereas the changes in earlier stages were not found (7, 8). After a direct irradiation (14.35 Gy) of the head of rats (8) the increase in the melatonin levels was recorded in later stages postirradiation period. No papers investigating the effect of ionizing radiation on the pineal catecholamines in laboratory rats have been found in the available literature. It has been known that catecholamines of the brain, hypothalamus, heart and other tissues are significantly decreased after exposure of ionizing irradiation (1, 6, 10—13) and that various changes occur in the early and prolonged period of postirradiation

(6). Based upon our results its follows, that the lethal protracted gamma-irradiation (6.78 Gy) markedly alters the hypothalamic catecholamine levels in the early postirradiation period (6, 14, 15). The whole-body irradiation of rats with X-rays injuries the metabolism and function of catecholamines in the whole brain, hypothalamus (14, 18) and adrenal glands and heart atria of rabbits and rats (7, 15). Stepanović *et al.* (10) found a marked decrease of catecholamines in the hypothalamus, corpus striatum and heart of rats after the whole-body irradiation with X-rays at a dose of 650 and 850 R.

The data on the effect of gamma-irradiation on the pineal catecholamines can be found only sporadically. In our previous work (6) after the whole-body, 5 days continous irradiation of sheep with daily dose of 0.5 Gy up to an accumulated dose of 2.50 Gy gamma-rays a significant decrease in the pineal norepinephrine and epinephrine levels ($p<0.001$), was found 120 h after exposure.

In this experiment a significant decrease in the pineal norepinephrine and dopamine levels in rats ($p<0.01$) was observed 30 and 120 minutes after exposure. One hour after irradiation the NE and DA levels did not differ from the control values. It is difficult to explain the increase in DA and NE levels 60 min after irradiation. It is possible that pineal gland, which is avoid of blood-brain-barrier, absorbs the necessary amount of catecholamines from the peripheral sources. A turnover and actual levels of catecholamines in the nerve tissue depend on many factors such as storage and uptake, absorption from transneural flux and interactions with autoreceptors (16). Injury of some of these factors with radiation leads to changes in the concentrations and functions of catecholamines in the nerve tissue.

The effects of prolonged ionizing radiation with a dose of 14.35 Gy on the pineal epinephrine levels were different. At 60 and 120 min after irradiation a decrease by 61.8% and 62.2%, respectively was recorded in comparison with the control group. The increase in epinephrine concentration immediately after exposure is probably the result of the stress effect of radiation (19).

Reduction of catecholamine levels in the rats pineal gland after exposure to lethal dose of the gamma-rays (14.35 Gy) is supposed to be related with impairment of the metabolic processes in the whole organism and endothelium of vessels as well as subsequent decrease or limitation of the synthesis of catecholamines. Stepanović *et al.* (10) found that irradiated rats with doses 650 and 850 R were able to metabolize L-DOPA a precursor of catecholamine synthesis and store the new-synthesized CA in the nerve tissue, when the activity of peripheral DOPA-decarboxylase was inhibited by benserazide. Thus, it is possible that X-irradiation of rats did not influence the mechanism of uptake and storage of norepinephrine and dopamine in the hypothalamus and other examined tissues. Furthermore, it is probable that the given changes could be also influenced by an increase in the activity of the enzymes responsible for catecholamine degradation observed after exposure to radiation in the brain of rabbits and ewes (11, 12, 14, 15).

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