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Reduced Pineal Melatonin Biosynthesis in Fractionally Irradiated Rats

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Summary

The effects of ionizing radiation on pineal melatonin and on key enzymes of its metabolism have been studied in our laboratory. After adaptation to an artificial light/dark cycle of 12:12 h, male Wistar rats were fractionally whole-body irradiated with a dose of 2.4 Gy of gamma-rays twice a week up to total doses of 4.8, 9.6 or 14.4 Gy. Irradiation and sham-irradiation were performed in the late afternoon. The rats were sacrificed at 24:00 to 01:00 h in darkness, 6 h, 3 or 5 days after the last exposure. Pineal and serum melatonin concentrations, pineal activities of serotonin N-acetyltransferase (NAT) and of monoamine oxidase (MAO) were determined. The NAT activities in the rats irradiated with 4.8 and 9.6 Gy decreased at some intervals without changes of melatonin concentration. Irradiation with a total dose of 14.4 Gy decreased NAT activity and the concentration of pineal and serum melatonin 6 h and 3 days after the last exposure. The activity of MAO, estimated only in the rats irradiated with the dose of 14.4 Gy, increased significantly 3 days after irradiation. The fractionated irradiation up to the dose of 14.4 Gy caused a transient decrease in pineal melatonin synthesis. This could be the consequence of preferential oxidative deamination of serotonin in comparison with its N-acetylation, leading to melatonin biosynthesis.

Key words
Fractionated irradiation – Rat pineal gland – Melatonin – Serotonin N-acetyltransferase – Monoamine oxidase

Introduction

The effects of ionizing radiation on pineal parameters in mammals or in man have not yet been explained sufficiently; in particular we lack data in laboratory rats subjected to fractionated irradiation. Previously published data about the effect of ionizing radiation on pineal parameters are sparse (Barfuss *et al.* 1969, Ellis *et al.* 1970). The response of important biochemical parameters in the pineal gland, melatonin and the rate-limiting enzyme of its synthesis, serotonin N-acetyltransferase, to ionizing radiation was investigated in our laboratory. Fractionated whole-body irradiation was chosen as a model for studying protracted effects of ionizing radiation, closely related to clinical practice (Ahlersová *et al.* 1997a).

Material and Methods

Male Wistar rats (Velaz, Prague) of 200 g body weight were adapted to an artificial light/dark cycle of 12:12 h under standard vivarium conditions (temperature 22±2 °C, relative humidity 60–70 %) for four weeks before irradiation. Cool light (fluorescent lamps Tesla, 40 W) of 150 lux intensity in each cage was switched on at 07:00. The rats had free acces to food and water. After adaptation, the animals were subjected to fractionated irradiation from a ^{60}Co

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source (Therapeutic apparatus Chisostat, exposure rate 0.29 Gy.min^{-1}). The doses of 2.4 Gy of gamma rays were applied twice a week on Tuesdays and Fridays up to total accumulated doses of 4.8, 9.6 or 14.4 Gy. Irradiation and sham-irradiation of the controls were performed at the end of the light part of day between 15:30 and 18:30 h. Irradiated and control rats were quickly decapitated in the dark 6 h, 3 and 5 days after the last exposure between 24:00 and 01:00 h in dim red light of less than 1 lux intensity. The pineal glands were weighed, frozen in liquid nitrogen and stored at −70 °C, the serum obtained from mixed blood was stored at −20 °C. The following parameters were determined: the concentration of melatonin radioimmunologically in the pineal gland and in the serum according to Charron *et al.* (1991), pineal serotonin N-acetyltransferase activity (NAT) radioenzymatically according to Deguchi and Axelrod (1972), in modification of Parfitt *et al.* (1975). The activity of monoamine oxidase (MAO) was estimated in the pineal gland radioenzymatically, according to Wurtman and Axelrod (1963), using ^{14}C-S-adenosyl-L-methionine as the common and specific substrate for MAO, type-A and type-B. The protein concentration in pineal homogenates was determined according to Lowry *et al.* (1951). The activity of MAO was analysed only on the 1st and 3rd day in rats irradiated with a total dose of 14.4 Gy. Each group consisted of 8–10 rats. The group differences were statistically analysed by the t-test.

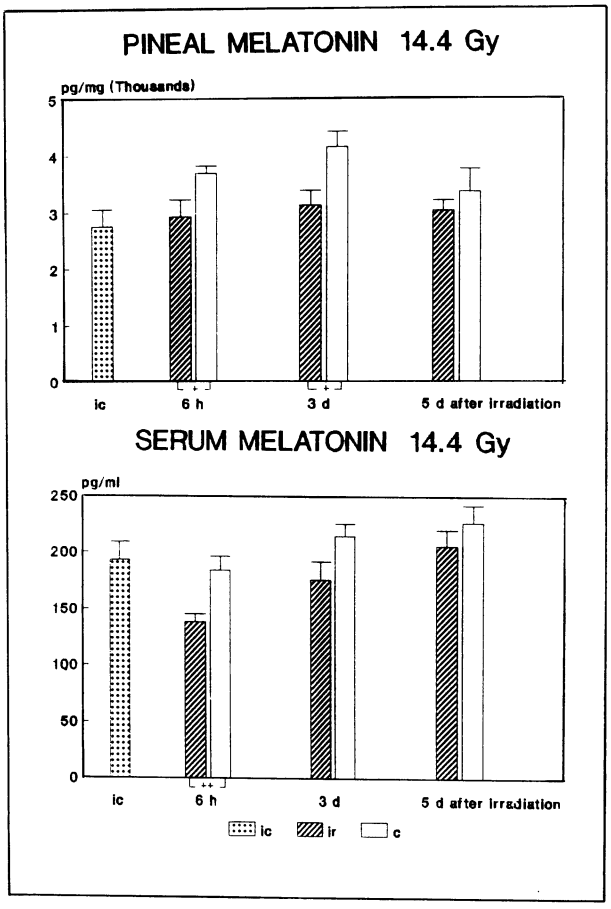

Fig. 1. *Pineal and serum melatonin concentrations in fractionally gamma irradiated rats – IR with 2 x 2.4 Gy of gamma rays weekly up to a total dose of 14.4 Gy, 6 h to 5 days after the last exposure. Sham-irradiated rats – C, intact rats – IC. Values are given as means ± S.E.M. The differences between groups are given as* $^{+}$ *P<0.05,* $^{++}$ *P<0.01.*

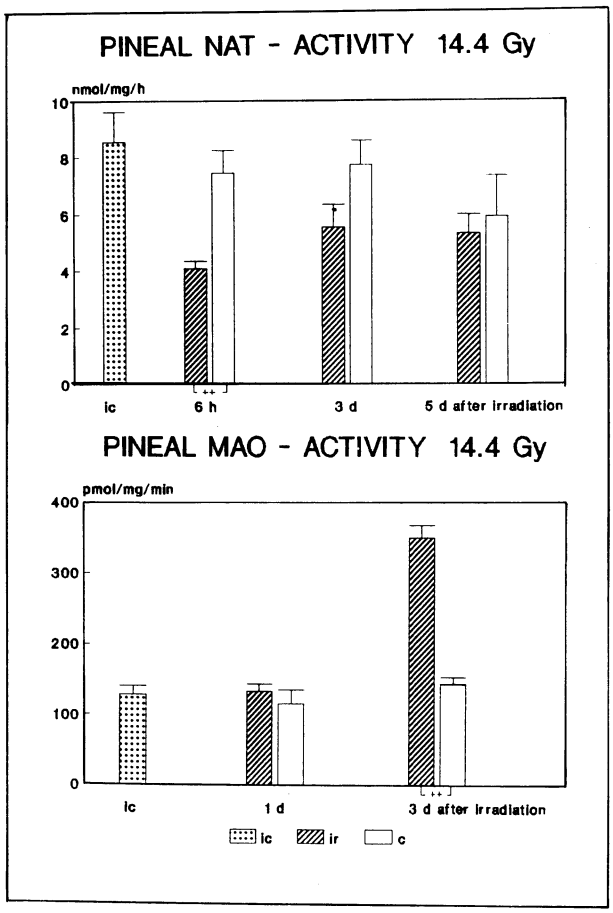

Fig. 2. *Pineal N-acetyltransferase (NAT) activity in fractionally irradiated rats 6 h to 5 days after the last exposure. Pineal monoamine oxidase (MAO) activity in rats 1 and 3 days after fractionated irradiation. Other details as in Figure 1.*

Results

The concentration of pineal and serum melatonin in the rats irradiated up to total doses 4.8 and 9.6 Gy did not differ from the control values (data not given). After the dose of 14.4 Gy, a significant decrease in concentration of pineal melatonin was noted at 6 h and 3 days intervals, the serum level of melatonin decreased 6 h postexposure (Fig. 1). A significant decrease in pineal NAT activity was observed in rats irradiated up to total doses of 4.8 Gy by day 5 (IR 6.06±0.63 vs C 8.02±0.54, $P<0.05$) and 9.6 Gy 6 h postexposure (IR 3.98±0.72 vs C 5.90±0.39, $P<0.05$). Irradiation up to a dose of 14.4 Gy significantly decreased NAT activity 6 h postexposure, a decrease on day 3 was of borderline significance. The activity of pineal MAO, determined only after irradiation with a dose of 14.4 Gy, exhibited no marked changes on the first day and was more than twice as high on the third day in comparison with the control values (Fig. 2).

Discussion

Melatonin in the pineal gland is synthesized from the amino acid tryptophan through serotonin, which is converted to N-acetylserotonin (a reaction catalyzed by serotonin NAT). Transformation of N-acetylserotonin to melatonin is catalyzed by hydroxyindole-O-methyltransferase (HIOMT). This metabolic pathway is activated in the dark part of the day (Mefford *et al.* 1983). The other major route of serotonin metabolism in the pineal gland, preferred under physiological conditions in the light part of the day, is the oxidative deamination catalyzed by monoaminooxidase A (Oxenkrug 1991), resulting in the formation of 5-hydroxyindole compounds (Mefford *et al.* 1983).

Single whole-body irradiation of rats with a dose of 14.4 Gy of gamma rays in our previous experiments decreased the concentration of pineal melatonin 30 to 120 min after the exposure; no changes in pineal NAT activity were noted. Three to four days after the exposure, signs of increased melatonin biosynthesis were observed in the pineal gland, i.e. a significant increase in NAT activity and serum melatonin concentrations (Kassayová *et al.* 1993, 1995, Ahlersová *et al.* 1997). These changes of pineal and serum melatonin concentrations found in our experiments are in good agreement with the initial decrease and later elevation of HIOMT pineal activity in single whole-body X-irradiated rats (Ellis *et al.* 1970).

In fractionally whole-body irradiated rats, the changes of pineal parameters followed were noted only at the highest accumulated dose of 14.4 Gy. The decrease in NAT activity and in melatonin concentration, and the increase in MAO activity up to 3 days after irradiation indicate that a reduction in melatonin biosynthesis had occurred in consequence of the changes in serotonin metabolism after irradiation. A decrease in serotonin concentration in the brain and other tissues lasting 6 days after X-irradiation of rats with a dose of 4.5 or 9.0 Gy was described by Ershoff *et al.* (1962). Elevated concentrations of serotonin metabolites (5-hydroxyindolyl compounds) were observed in the brain of laboratory rats early after X-irradiation with a dose of 9.0 Gy (Palaic *et al.* 1964). Ionizing radiation increased MAO activity in the brain of rabbits (Pausescu *et al.* 197) and of sheep (Pástorová and Arendarčík 1988). The decrease in pineal melatonin concentration in fractionally gamma irradiated rats or/and shortly after exposure to a single lethal dose of gamma rays (Kassayová *et al.* 1995) might be the consequence of preferred oxidative deamination of serotonin in comparison with the N-acetylation route of melatonin biosynthesis. For the final confirmation of our hypothesis the determination of 5-hydroxyindole derivates in the pineal gland of irradiated rats should be performed.

The transient decrease of melatonin synthesis in fractionally irradiated rats in our paper concerns the enhanced sensitivity of the pineal gland to repeated irradiation. Pubertal girls in complete remission of acute lymphoblastic leukaemia, 5 years after cranial irradiation with an accumulated dose 21.6 Gy of gamma rays, exhibit significantly lower melatonin plasma concentrations in comparison with healthy controls (Commentz and Damman 1995). The decrease of melatonin synthesis could change various functions regulated by the pineal gland, namely immunological functions and antioxidant activity in irradiated organisms.

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