Physiol. Res. 48: 227-230, 1999

Two-Phase Response of Rat Pineal Melatonin to Lethal Whole-Body Irradiation With Gamma Rays

M. KASSAYOVÁ, E. AHLERSOVÁ, I. AHLERS

Institute of Animal Physiology, Faculty of Science, P.J. Šafárik University, Košice, Slovak Republic

Received October 21, 1997
Accepted February 16, 1999

Summary

Male Wistar rats adapted to artificial light:dark (LD) regimen 12:12 h were whole-body irradiated with a single dose of 9.6 Gy of gamma rays and sham/irradiated in the night in darkness. The rats were examined 60 min, 1, 3 and 5 days after exposure between 22:00 and 01:30 h in the darkness. The results obtained indicate a two-phase reaction of pineal melatonin after the lethal irradiation of rats: the decline of melatonin concentration early after the exposure (at 60 min) with unchanged serotonin N-acetyltransferase (NAT) activity followed by an increase of melatonin synthesis, accompanied by an increase of pineal and serum melatonin on day 5 after the exposure. NAT activity was increased on day 3 after the exposure. Serum corticosterone concentrations in irradiated rats were increased 60 min and 3 days after exposure. With respect to the antioxidant, immunomodulating and stress-diminishing properties of melatonin, we consider the increase in melatonin synthesis during later periods after irradiation as part of adaptation of the organism to overcome radiation stress.

Key words

Pineal melatonin • Serotonin N-acetyltransferase activity • Ionizing radiation • Rats

Introduction

With respect to the significance of the pineal gland and its main hormone melatonin (Mel) in regulation of various physiological processes (Reiter 1991), information about the effect of ionizing radiation on this gland is very important both in basic research and especially in clinical practice, namely in patients treated by actinotherapy. In our previous experiments we studied the effects of different doses of gamma radiation on Mel concentration and activity of the rate limiting enzyme of its synthesis, serotonin N-acetyltransferase (NAT) in the pineal gland. Three model doses of radiation were chosen: 14.4, 9.6 and 4.8 Gy of gamma rays. Acute whole-body irradiation with the lowest of these doses (4.8 Gy) did not change either NAT activity or Mel concentration in the pineal gland (Kassayová, unpublished results). Exposure to the 14.4 Gy caused marked changes in Mel concentration, depending on the time interval after irradiation (Kassayová *et al.* 1993a, 1995). The reason for using the lowered lethal dose (9.6 Gy) in this experiment was to create the possibility of prolonging the time for observation; the animals began to die on day 10 postexposure as compared with day 5 after irradiation with a dose of 14.4 Gy.

Fax +420 2 293 894
E-mail physres@biomed.cas.cz http://www.lf2.cuni.cz/physiolres/physiolres.htm

Method

Male Wistar rats (VELAZ Prague), weighing about 100 g, were adapted to an artificial light-dark regimen (12 h light : 12 h darkness) for 6 weeks under standard vivarium conditions (temperature 22±2 °C, relative humidity 60-70 %). Cool light from fluorescent lamps (Tesla 40 W), about 150 lux intensity in each cage, was automatically switched on at 07:00 h. The animals had free access to food and water. After adaptation, the rats were whole-body irradiated with a single dose of 9.6 Gy of gamma rays from a ^{60}Co source (Chisostat, exposure rate 0.38 Gy.min^{-1}) between 20:30 and 00:20 h. After irradiation and sham-irradiation the animals were pair-fed till the estimation. Intact rats (IC) were fed *ad libitum*. Sixty minutes 1, 3 and 5 days after irradiation or sham-irradiation the rats were sacrificed by rapid decapitation between 22:00 and 01:30 h (the group of intact rats was killed between 23:45 and 24:00). Each rat was killed within 10 s. The real time-difference between killing the animals of sham-irradiated and irradiated groups was about 20 min. Irradiation, sham-irradiation and decapitation were carried out in the dark under dim red light of less than 1 lux intensity. The pineal glands were rapidly removed, weighed, frozen in liquid nitrogen and stored at −70 °C until further analysis. The serum obtained from mixed blood was immediately frozen and stored at −20 °C. The experiment was carried out in January.

The concentration of pineal and serum melatonin was assessed radioimmunologically according to Charron *et al.* (1991). The pineal NAT activity was determined radioenzymatically according to Deguchi and Axelrod (1972) as modified by Parfitt *et al.* (1975). The serum corticosterone (CS) concentration was determined fluorometrically according to Guillemin *et al.* (1958). Each group consisted of 8 rats, and the results were evaluated using the non-paired t-test.

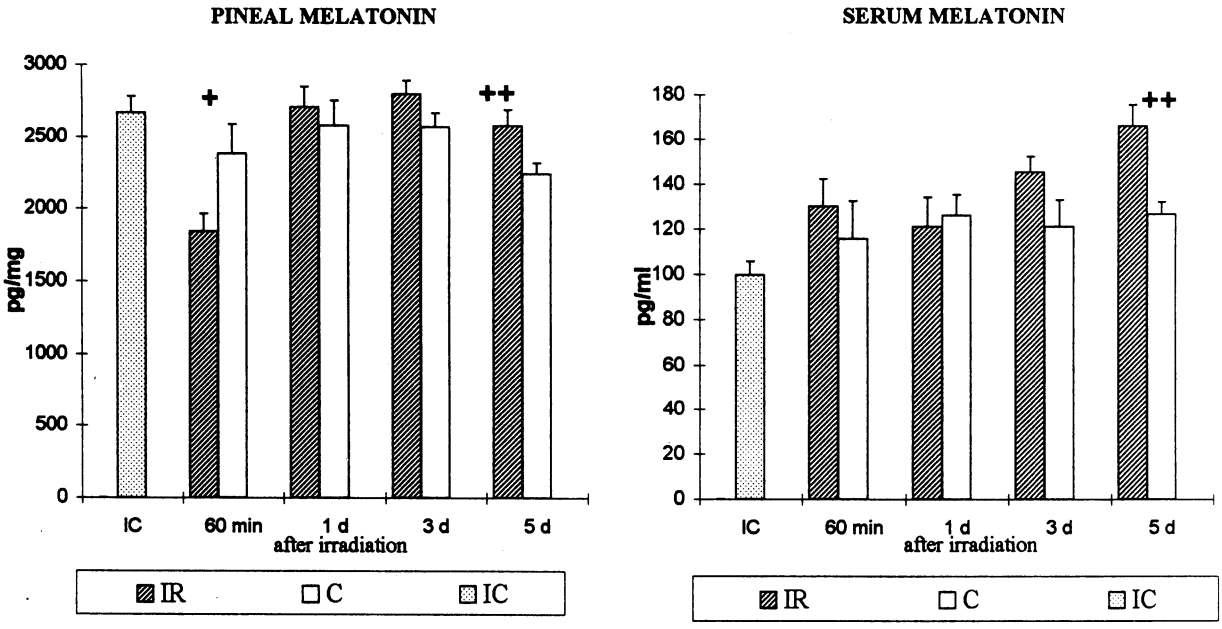

Fig. 1. *Pineal and serum melatonin concentrations in irradiated (IR) and sham-irradiated (C) rats after single whole-body irradiation with a dose of 9.6 Gy of gamma rays. Irradiated (IR) and sham-irradiated (C) rats were analyzed in darkness from 60 min to 5 days after irradiation, intact rats (IC) – values obtained in darkness. Values are means ± S.E.M, n = 8 (+ P<0.05 and ++ P<0.01, IR vs C).*

Results

The changes in concentration of Mel in the pineal gland of irradiated animals occurred in two phases: an initial decrease at 60 min (P<0.05) was followed by an increase in values, significant on day 5 post-irradiation (P<0.01) in comparison with the controls. A significant increase in concentration of serum Mel was noted on day 5 after exposure (P<0.01, Fig. 1). The activity of pineal NAT exhibited an increase on day 3 after exposure (P<0.01), without any changes in the remaining intervals of observation. The level of CS in the serum of irradiated

rats increased significantly at 60 min ($P<0.01$) and was approximately five times higher on day 3 in comparison with the control values ($P<0.01$, Table 1). Neither the absolute nor relative average weights of the pineal gland were affected by irradiation.

Table 1. Pineal serotonin N-acetyltransferase (NAT) activity and serum corticosterone concentration in irradiated and sham-irradiated rats after single whole-body irradiation with a dose of 9.6 Gy of gamma rays

			60 min	1 day	3 days	5 days
Pineal NAT	IC	5.84± 1.02	–	–	–	–
activity	IR		5.66±0.52	5.17±0.49	8.21±0.61^{++}	5.05±0.26
(nmol/h/mg)	C		6.24±0.80	6.66±0.97	5.45±0.49	6.65±0.44
Serum	IC	171.0± 38.0	–	–	–	–
corticosterone	IR		592.3±58.7^{++}	440.6±61.9	1056.0±73.5^{++}	227.8±43.4
(pmol/ml)	C		274.5±64.9	339.6±49.4	209.5±44.2	245.7±64.1

Values are means ± S.E.M, n=8 ($^{++}$ $P<0.01$, IR vs C).

Discussion

The response of the pineal gland to whole-body irradiation with a dose of 9.6 Gy consisted in two-phase changes in melatonin concentration. Immediately after exposure the concentration decreased, but after a few days (on day 5) it increased. NAT activity increased 3 days after exposure. A similar trend in Mel concentration and NAT activity was found in rats irradiated with a single whole-body lethal dose of 14.4 Gy of gamma rays in the previous experiments. Up to 2 h after exposure, pineal Mel concentrations decreased, however, on days 3 and 4 signs of increased Mel synthesis were observed, with a rise of pineal NAT activity and serum Mel concentration (Kassayová *et al.* 1993a, 1995, Ahlersová *et al.* 1997). In rats locally irradiated on the head with a dose of 14.4 Gy of gamma rays the pineal Mel content and its serum concentration increased only on days 1-3 after exposure, with a return to control values within 10 days; the Mel content was not changed immediately after the exposure (Kassayová *et al.* 1993b).

Serum corticosterone (CS) reacted to acute whole-body irradiation with a dose of 9.6 Gy by an increased concentration 60 min and 3 days after exposure. This finding is in agreement with the classical concept of Flemming *et al.* (1968) according to which the response of CS to whole-body irradiation occurs in two phases: the first peak represents an early response to the radiation stressor and the second peak, the onset of which depends on the radiation dose and which corresponds to the patterns of gastrointestinal damage in the acute phase of the postradiation syndrome.

The mechanism by which ionizing radiation influences pineal function and its main hormone Mel still remains to be explained. Under physiological conditions, Mel synthesis exhibits a circadian rhythm with low values of hormone concentrations during the light part of the day and high values in darkness. Two reciprocal metabolic pathways of pineal serotonin are connected with this circadian rhythm. In the light part of the day, oxidative deamination of pineal serotonin catalyzed by monoaminooxidase (MAO) with the production of 5-hydroxyindole derivatives and low Mel synthesis. In the dark part of day, the acetylation of serotonin with high activity of NAT and increased Mel production are activated (Mefford *et al.* 1983). The increased MAO activity, which was observed in the brain of X- or gamma-irradiated animals (Pausescu *et al.* 1976, Pástorová and Arendarčík 1988), leads to a preference of serotonin degradation to 5-hydroxyindole products, which may be a cause of the decline of Mel concentrations immediately after irradiation, found in our studies. A different situation occurred in male rats subjected to repeated (fractionated) irradiation with 2.4 Gy of gamma rays twice a week. The concentration of pineal and serum melatonin decreased,

pineal NAT activity was reduced and pineal MAO activity was enhanced in animals exposed to the highest (14.4 Gy) total dose of radiation (Ahlersová *et al.* 1998).

The increase of pineal Mel synthesis during the later period after whole-body exposure to gamma rays could be considered as an adaptive effort of the organism to overcome the radiation stress by an increased production of the hormone with immunomodulating and antioxidative efficacy.

Acknowledgments

The project No. 1/1236/94 was supported by the Grant Science Agency of the Slovak Republic. The experiments were conducted according to the principles provided in the Law No 115/1995 §24 of the Slovak Republic for the Care and Use of Laboratory Animals.

References

AHLERSOVÁ E, KASSAYOVÁ M, AHLERS I: Serum melatonin, corticosterone and thyroid hormones in irradiated rats: effect of exposure during various times of day and of light regimen: *J Physiol Pharmacol* **48:** 435-442, 1997.

AHLERSOVÁ E, PÁSTOROVÁ B, KASSAYOVÁ M, AHLERS I, ŠMAJDA B: Reduced pineal melatonin biosynthesis in fractionally irradiated rats. *Physiol Res* **47:** 133-136, 1998.

CHARRON G, SOULOUMIAC I, FOURNIER MC, CANIVENC R: Pineal rhythm in N-acetyltransferase activity and melatonin in the male badger Meles meles L. under natural daylight: relationship with the photoperiod. *Pineal Res* **11:** 80-85, 1991.

DEGUCHI T, AXELROD J: Sensitive assay for serotonin N-acetyltransferase activity in rat pineal. *Anal Biochem* **50:** 174-179, 1972.

FLEMMING K, GEIERHAAS B, HEMSING W, MANNIGEL U: Strahlenwirkungen und Nebennierenrinde. IV. Untersuchungen an Ratten über den ersten Corticosteroidanstieg nach Ganzkörper-Röntgenbestrahlung. *Int. Radiat Biol* **14:** 93-105, 1968.

GUILLEMIN R, CLAYTON G.W, SMITH J.D, LIPSCOMB HS: Measurement of free corticosteroids in rat plasma. Physiological validation of a method. *Endocrinology* **63:** 349-355, 1958.

KASSAYOVÁ M, AHLERSOVÁ E, AHLERS I: Changes of pineal N-acetyltransferase activity in gamma irradiated rats. *Physiol Res* **42:** 167-169, 1993a.

KASSAYOVÁ M, AHLERSOVÁ E, AHLERS I, MILÁROVÁ R: The changes in pineal N-acetyltransferase activity, pineal and serum melatonin concentration in rats after irradiation of the head. *Physiol Res* **42:** 419-422, 1993b.

KASSAYOVÁ M, AHLERSOVÁ E, PÁSTOROVÁ B, AHLERS I: The early response of pineal N-acetyltransferase activity, melatonin and catecholamine levels in rats irradiated with gamma rays. *Physiol Res* **44:** 315-320, 1995.

MEFFORD IN, CHANG P, KLEIN DC, NAMBOODIRI MA, SUGDEN D, BARCHAS J: Reciprocal day/night relationship between serotonin oxidation and N-acetylation products in the rat pineal gland. *Endocrinology* **113:** 1582-1586, 1983.

PARFITT A, WELLER JL, KLEIN DC: Blockade by ouabain or elevated potassium ion concentration of the adrenergic and adenosine cyclic 3′,5′-monophosphate-induced stimulation of pineal serotonin N-acetyltransferase activity. *Mol Pharmacol* **11:** 241-255, 1975.

PÁSTOROVÁ B, ARENDARČÍK J: The effect of protracted exposure to gamma radiation (6.7 Gy) on the activity of monoaminooxidase in the hypothalamus of ewes in the anoestral period (in Slovak). *Vet Med (Praha)* **33:** 735-740, 1988.

PAUSESCU E, POPESCU M, PAUN M, TEODOSIU T: Dynamics of the changes in the tissue levels of cyclic AMP after ^{60}Co gamma irradiation. *Strahlenterapie* **151:** 165-171, 1976.

REITER R.J: Melatonin: that ubiquitously acting pineal hormone. *News Physiol Sci* **6:** 223-227, 1991.

Reprint requests

M. Kassayová, Institute of Animal Physiology, Faculty of Science, P.J. Šafárik University, Moyzesova 11, 041 67 Košice, Slovak Republic, E-mail: kassaym@kosice.upjs.sk