

The Changes in Pineal N-Acetyltransferase Activity, Pineal and Serum Melatonin Concentration in Rats After Irradiation of the Head

M. KASSAYOVÁ, E. AHLERSOVÁ, I. AHLERS, R. MILÁROVÁ

Institute of Animal Physiology, Šafárik University, Košice, Slovak Republic

Received April 28, 1993

Accepted August 31, 1993

Summary

Male Wistar rats adapted to a light/dark cycle (LD) 12:12 h were exposed in the darkness to a single dose of 14.35 Gy gamma rays on the head with the body shielded. Irradiated and sham-irradiated rats were kept again in the 12 h LD cycle with a free access to food and water till the analysis performed in the darkness. Pineal N-acetyltransferase activity and melatonin content, the serum concentration of the melatonin, corticosterone, thyrotropin and thyroid hormones were determined. N-acetyltransferase activity was lower 2–24 h after irradiation non-significantly whereas between 3–10 days it did not differ from the controls. Radiation decreased the pineal melatonin content and its serum concentration 2 h after exposure and increased them significantly 1–3 days after irradiation. No changes in melatonin levels were found on postirradiation days 5–10. The corticosterone concentration was increased 2 h after exposure only. Local head irradiation changed neither thyrotropin nor thyroid hormone levels.

Key words

Head gamma irradiation – Rats – Pineal N-acetyltransferase activity – Pineal melatonin content

Introduction

Information about the effects of ionizing radiation on the pineal gland is of importance not only for basic research but mainly for clinical practice in patients treated by radiotherapy. The production of melatonin, the main pineal hormone, was found to be rhythmical with high levels in the darkness and low in the light. It is regulated by sympathetic nerve fibres, that influence beta- and alpha-adrenergic receptors of pinealocytes via norepinephrine. We have recently reported the changes of pineal N-acetyltransferase activity (NAT) in rats exposed to a single whole-body dose of 14.35 Gy gamma rays either in the light or in the dark part of the day (Kassayová *et al.* 1993). In the present paper we studied the effects of local irradiation of the head on the pineal gland and also on some hormone levels.

Material and Methods

Male Wistar rats weighing about 180 g were adapted to a light-dark regimen with 12 h of light and 12 h of darkness per day (LD 12:12) for two weeks under standard vivarium conditions (22 ± 2 °C, relative humidity 60–70 %) in October. Cool light (fluorescent lamps Tesla, 40 W) of 150 lux intensity per cage was automatically switched on at 0700 h. The rats had free access to water and food. After this adaptation the fed rats were exposed to a single dose of 14.35 Gy of gamma rays locally on the head from a ^{60}Co source (therapeutic apparatus Chisostat, exposure rate 0.38 Gy/min under pentobarbital anaesthesia (40 mg/kg of weight, subcutaneously) in special perspex boxes described by Skopec (1986) in our own modification. The frontal lead side of the box that was 8 mm thick (lead "collar") fixed and shielded the body in the neck region. The other parts were shielded by a lead shelter of 100 mm thickness from above, 50 mm from the sides

and back and 25 mm from below. This shielding ensured a sharp fall of the dose; 1 cm from the box face the dose corresponded to 100 % (14.35 Gy), while 1 cm behind it the dose fell to 2.2 %, and to 1.8 % in the most remote parts. Two boxes were placed opposite each other so that the heads of rats were in the isodose with 99 % potency of the source. The doses were measured by thermoluminescent dosimeters [^7LiF], and evaluated by Victoreen TLD Reader 2800. The controls were sham-irradiated by the same procedure. Irradiated and sham-irradiated rats were kept in the light/dark regimen 12:12 h with free access to food till analyzed. Irradiation and sacrificing of the rats were carried out in the darkness (using a dim red light of the intensity less than 1 lux) between 2300–2400 h, except of the groups analyzed 2 h after exposure which were irradiated between 2100–2200 h and sacrificed

between 2300–2400 h. NAT activity was determined in the pineal gland radioenzymatically according to Deguchi and Axelrod (1972) with the modification according to Parfitt *et al.* (1975). Pineal and serum melatonin was determined radioimmunochemically according to Charon *et al.* (1991). The serum corticosterone concentration was determined fluorimetrically (Guillemin *et al.* 1958), thyrotropin (TSH) and thyroid hormones concentration radioimmunologically (NIDDK rat TSH-RIA kit from National Hormone Pituitary Program, University of Maryland, School of Medicine, USA; total T_4 and T_3 RIA kits from the Institute for Research, Production and Application of Radioisotopes, Prague, Czech Republic). Each group consisted of 6 rats. The results were evaluated using the non-paired t-test.

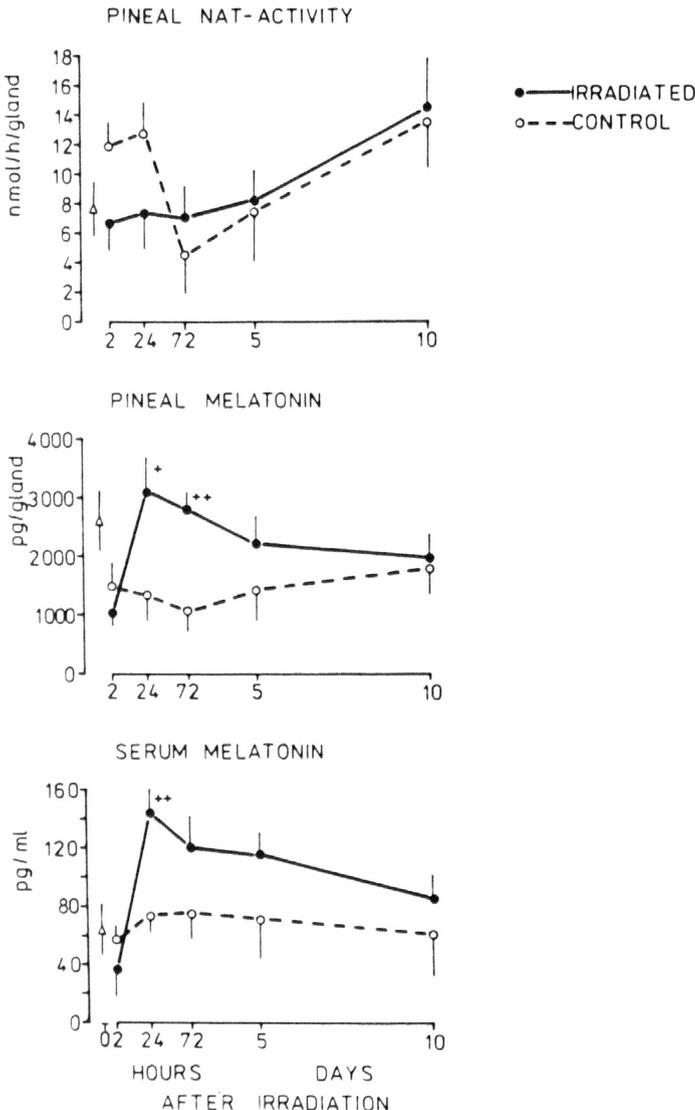


Fig. 1

Pineal N-acetyltransferase (NAT) activity, pineal melatonin content and serum melatonin concentration in rats after irradiation and sham-irradiation (control) of the head, at various intervals after the exposure. Rats were irradiated and analysed in the darkness. Values are given as Means \pm S.E.M. Triangles represent values of intact rats. Differences between groups are given as + $P < 0.05$, ++ $P < 0.01$.

Results

The pineal NAT activity was decreased, though statistically non-significantly, within the first 2–24 h after irradiation, but 3–10 days later it did not differ from the controls. The pineal melatonin content slightly decreased 2 h after irradiation, rose markedly on days 1–3, before falling to the level of non-irradiated controls on days 5–10 after the exposure.

The serum melatonin concentration in irradiated animals had a similar pattern of changes as in the pineal gland (Fig. 1.).

The serum corticosterone concentration (B) was increased 2 h after exposure, but then the values were similar to those of the controls. The irradiation changed neither TSH nor thyroxine (T₄) and triiodothyronine (T₃) concentrations in the serum (Table 1).

Table 1
The concentration of serum corticosterone (B), thyrotropin (TSH), thyroxine (T₄) and triiodothyronine (T₃) in rats after irradiation (IR) and sham-irradiation (C) of the head

Hours/Days after irradiation		2 h	24 h	3 d	5 d	10 d
B pmol/ml	IR	693.0±123.0 ⁺⁺	497.0±88.1	359.0±91.4	458.0±95.1	306.0±52.7
	C	214.0±69.0	526.0±75.4	341.0±73.3	282.0±92.8	284.0±32.1
TSH µg/l	IR	0.8±0.1	2.5±0.4	2.3±0.7	2.3±0.3	1.5±0.1
	C	1.0±0.1	3.7±0.5	1.5±0.3	2.6±0.5	2.0±0.5
T ₄ nmol/l	IR	74.3±6.5	74.8±8.4	79.8±7.2	87.8±2.9	84.8±4.6
	C	80.5±8.4	92.0±7.3	65.3±8.3	94.6±4.7	92.0±6.3
T ₃ nmol/l	IR	1.0±0.1	0.7±0.1	0.8±0.2	0.9±0.1	0.8±0.1
	C	0.9±0.1	0.8±0.1	0.6±0.1	0.9±0.1	1.0±0.1

The values are given as Means ± S.E.M., (n=6), ⁺⁺ significantly different from controls (P<0.01)

Discussion

Until recently, only two papers referring the effects of ionizing radiation on the pineal functions in laboratory rodents were available. They reported the activity of pineal hydroxyindole-O-methyltransferase (HIOMT), the enzyme catalyzing the transformation of N-acetylserotonin to melatonin, in whole body irradiated male rats. Barfuss *et al.* (1969) reported a decrease in HIOMT activity 24 h after irradiation of rats with 450 R of X-rays. Ellis *et al.* (1970) found a decrease in HIOMT activity in rats irradiated with 350 R of X-rays within 1–24 h after exposure. A subsequent increase between days 4–18 returned to control values later. The changes in NAT activity and the pineal melatonin content after a local irradiation of the head observed in our experiment were diversified with the exception of the early postirradiation response (2 h after exposure), when the values of both parameters were found to be decreased. NAT activity still remained lower 24 h after irradiation but between days 3–10 it did not differ from the controls. Increased N-acetyltransferase activity in control rats after 2 h and

24 h might be caused by pentobarbital anaesthesia; an effect absent in irradiated rats. The NAT activity in both irradiated and nonirradiated animals corresponded, in general, to values of intact, non-treated rats during the whole observed period. Pineal and serum melatonin responded to the ionizing radiation in a biphasic manner. After the initial decrease, it steeply rose between 24 h and 72 h after irradiation and then gradually decreased to control values. The increase in pineal melatonin content 24 h and 72 h after irradiation might be caused by enhanced offer of serotonin and/or tryptophan released following irradiation. The artificial magnetic field decreased NAT activity and the melatonin content in the pineal gland 2 h after the exposure (Welker *et al.* 1983), similarly as ionizing radiation. This was, however, non-significant in our experiment. In male rats whole-body irradiated with the dose of 14.35 Gy gamma rays, we recorded an increase of pineal NAT activity on 3–4 days, without any changes at earlier intervals after the exposure (Kassayová *et al.* 1993).

The pineal response to ionizing radiation is probably also mediated by changes of adrenergic

nervous system activity, which plays a direct role in the regulation of melatonin synthesis, but the data on norepinephrine levels in the pineal gland or on adrenergic receptors of pinealocytes after irradiation are still lacking. One day after irradiation of the rats with 8.5 Gy of X-rays, the level of norepinephrine in the brain was reduced by 60 % (Varagic *et al.* 1967), but the level of circulating catecholamines was not changed in rats exposed to either lethal or sublethal doses of X-rays (Griffith *et al.* 1961). We found an early response to the radiation stressor in serum corticosterone levels, which were increased markedly as early as 2 h after irradiation. *In vitro*, corticosterone significantly reduced norepinephrine and cAMP-stimulated melatonin levels in the pineal gland and in the incubation medium, but it did not influence the basal hormone level (Fevre-Montange and Abou-Samra 1983). Local irradiation of the head in our

experiment influenced neither T₄ nor T₃ concentrations (the thyroid gland was well shielded from direct effects of radiation) nor the TSH concentration. After whole-body irradiation with doses ranging from 7.17 to 14.35 Gy of gamma rays, the T₄ and T₃ levels were markedly changed (Ahlersová *et al.* 1988). In further studies, an early NAT and melatonin response within 2 h after exposure to whole body irradiation will be investigated along with pineal catecholamine levels.

Acknowledgements

This study was supported by a grant No 2-990719-92 of Ministry of Education and Science, Slovak Republic. We wish to thank S. Raiti, M.D., F.A.C.P. for rat TSH RIA components from the NIDDK and NHPP Program, University of Maryland, School of Medicine, USA.

References

- AHLERSOVÁ E., MOLČANOVÁ A., AHLERS I.: Thyroid hormones in gamma irradiated rats. *Radiobiol. Radiother.* **29**: 507–512, 1988.
- BARFUSS G., TAIT G.R., ELLIS L.C.: Melatonin synthesis in rat pineal gland inhibited by X-irradiation. *Proc. Soc. Exp. Biol. Med.* **131**: 35–36, 1969.
- CHARRON G., SOULOUMIAC I., FOURNIER M.C., CANIVENC R.: Pineal rhythm of N-acetyltransferase activity and melatonin in the male badger *Meles meles L.* under natural daylight: relationship with the photoperiod. *J. Pineal. Res.* **11**: 80–85, 1991.
- DEGUCHI Z., AXELROD J.: Sensitive assay for serotonin N-acetyltransferase activity in rat pineal. *Analyt. Biochem.* **50**: 174–179, 1972.
- ELLIS L.C., BARFUSS D.W., VAN KAMPEN K.R.: Modification of rat testicular response to X-irradiation by various lighting regimens. 1. The pineal gland and melatonin synthesis as a possible mediator of the response. *Life Sci.* **9**: 1011–1020, 1970.
- FEVRE-MONTANGE M., ABOU-SAMRA A.B.: Glucocorticoids inhibit the in vitro melatonin production by rat pineal stimulated by norepinephrine (NE) or 8-bromo-cyclic AMP. *Neuroendocrinol. Lett.* **5**: 403, 1983.
- GRIFFITH M.G., GRIFFITH J.Q., HERMEL M.B., GERSHON-COHEN J.: Effect of irradiation to adrenals upon circulating adrenal medullary hormone in the rat. *Radiology* **77**: 91–93, 1961.
- GUILLEMIN R., CLAYTON G.W., SMITH J.D., LIPSCOMB H.S.: Measurement of free corticosteroids in rat plasma. Physiological validation of a method. *Endocrinology* **63**: 349–355, 1958.
- KASSAYOVÁ M., AHLERSOVÁ E., AHLERS I.: The changes of pineal N-acetyltransferase activity in lethally gamma irradiated rats. *Physiol. Res.* **42**: 167–169, 1993.
- PARFITT A., WELLER J.L., KLEIN D.C.: 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.
- SKOPEC F.: A method for head irradiation in the rats (in Czech). *Coll. Sci. Commun. Purkyně Med. Res. Inst. Hradec Králové* **101**: 169–175, 1986.
- VARAGIC V., STEFANOVIC S., SVECENSKI N.: The effect of X-irradiation on the amount of catecholamines in heart atria and hypothalamus of the rat. *Int. J. Radiat. Biol.* **12**: 113–119, 1967.
- WELKER H.A., SEMM P., WILLING R.P., COMMENTZ J.C., WILTSCHKO W., VOLLRATH L.: Effects of an artificial magnetic field on serotonin N-acetyltransferase activity and melatonin content of the rat pineal gland. *Exp. Brain Res.* **50**: 426–432, 1983.

Reprint Requests

Dr. M. Kassayová, Institute of Animal Physiology, Faculty of Science, Šafárik University, 041 67 Košice, Moyzesova 11, Slovak Republic.