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The Early Response of Pineal N-acetyltransferase Activity, Melatonin and Catecholamine Levels in Rats Irradiated with Gamma Rays

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Summary

Male Wistar rats adapted to an artificial light-dark regimen (12 h light : 12 h darkness) were whole-body irradiated with a dose of 14.35 Gy of gamma rays. Irradiation, sham-irradiation and decapitation 30, 60 and 120 min after the exposure were performed between 2000 h and 0100 h in the darkness. The serotonin N-acetyltransferase activity (NAT), the concentration of melatonin, dopamine, norepinephrine and epinephrine were measured in the pineal gland. The serum levels of melatonin and corticosterone were also determined. Ionizing radiation did not change the activity of the key enzyme of melatonin synthesis, NAT, but decreased the concentration of pineal melatonin. The concentration of pineal dopamine and norepinephrine decreased 30 and 120 min after exposure, while the concentration of epinephrine was elevated 30 min after irradiation, though later it was markedly decreased. The serum melatonin level was not changed, but an increase in corticosterone level was observed. In the early period after the exposure, a decrease in pineal melatonin occurred, accompanied by a decrease in pineal catecholamines. On the contrary, in the phase of developed radiation injury the signs of increased melatonin synthesis were observed on days 3 and 4 after the exposure (Kassayová *et al.* 1993a). The underlying mechanisms require further research.

Key words

Pineal gland – Melatonin – Catecholamines – Ionizing radiation – Rats

Introduction

Knowledge about the pineal gland reaction to ionizing radiation is very important not only in the field of basic research, where this problem has rarely been investigated, but especially in clinical practice. The therapeutic irradiation of cancer patients when directed to the head and neck regions can seriously influence their neuroendocrine functions. It is surprising how little is known about the pineal reaction to whole-body and local irradiation of the head. By its neuroendocrine multi-potential (Reiter 1991), the affection of pineal functions can modify the course of radiation effect on the central nervous system as well as on the whole organism.

The production of main pineal indoleamine, melatonin, is rhythmical, high during darkness and low during light. Melatonin synthesis is regulated by sympathetic fibres influencing the beta- and alpha-adrenergic receptors in the pinealocyte membrane by norepinephrine (Sugden 1989). There are several papers dealing with morphological changes in the pineal gland after whole-body and local irradiation of laboratory animals. Bayer *et al.* (1970) irradiated female Wistar rats pansomatically with the head shielded, and locally just the head with the body shielded with a dose of 700 R of X-rays. Analysis of the pineal ultrastructure 24 h and 2 and 6 days after the exposure revealed symptoms of pinealocyte impairment or activation. The pericapillary spaces were enlarged, while nerve endings were not affected. After local irradiation of the head, the changes were more

marked and appeared earlier. Two earlier papers investigating the activity of the terminal enzyme of melatonin synthesis, hydroxyindole-O-methyltransferase (HIOMT) reported changes in melatonin synthesis in irradiated rats. Barfuss *et al.* (1969) recorded a decrease in HIOMT activity 24 h after exposure of rats to 450 R of X-rays. Ellis *et al.* (1970) found a decrease of HIOMT activity in rats irradiated with 350 R of X-rays within 1–24 h after the exposure whereas at later periods after irradiation (between days 4–19) the enzyme activity was increased. Experiments were carried out under conditions of natural light, the time of irradiation and analysis were not given.

No papers investigating the effects of ionizing radiation on the pineal catecholamines in laboratory rats have been found in the available literature. A marked decrease of catecholamines in the hypothalamus and heart atria of rabbits, and in the whole brain and heart of rats was found 24 h after whole-body irradiation with doses of 1000 R or 850 R of X-rays (Varagic *et al.* 1967). Furthermore, Stepanovic *et al.* (1980) found that X-irradiation of rats with doses of 650 or 850 R did not influence the mechanism of uptake and storage of norepinephrine and dopamine in the hypothalamus and other examined tissues. Ionizing radiation changed the activity of the enzymes responsible for catecholamine degradation in the brain of rabbits and ewes (Pausescu *et al.* 1976, Pástorová and Arendarčík 1988).

The effect of single whole-body irradiation with 14.35 Gy gamma rays on the activity of serotonin N-acetyltransferase (NAT), a key enzyme of melatonin synthesis in the pineal gland, had been investigated in laboratory rats (Kassayová *et al.* 1993a). An increase in NAT activity during the period of manifested radiation injury was observed between 3–4 days after the exposure, without any changes in enzymatic activity at the earlier period (6–24 h after exposure). In this paper, we focus on the early postirradiation examination of more parameters of pineal activity in the same experimental model.

Methods

Male Wistar rats, weighing 200 g, were adapted to an artificial light-dark regimen (12 h light : 12 h darkness) for six weeks under standard 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 0700 h. The rats had free access to water and food (ST pellets, Velaz, Prague) until irradiation. Whole-body irradiation with a dose of 14.35 Gy gamma rays from a ^{60}Co source (Therapeutic Apparatus Chisostat, exposure rate 0.38 $Gy.min^{-1}$) as well as sham-irradiation (controls) were performed between 2000 h and 0100 h. Thirty, 60 and 120 min after the radiation exposure or sham-exposure the rats were quickly decapitated between 2040 and 0130 h. The irradiation, sham-irradiation and decapitation were carried out in darkness under dim red light of less than 1 lux intensity. Since it was impossible to determine all the parameters in a single pineal gland, the irradiation and examination were performed on two consecutive days: on the first day the pineal glands were used for analysis of NAT and melatonin, on day 2 for determination of catecholamines. The pineal glands were rapidly removed, weighed, frozen in liquid nitrogen and stored at −70 °C until further analysis. The serum obtained from the mixed blood was immediately frozen and stored at −20 °C until determination of melatonin and corticosterone concentrations. The experiment was carried out in May 1993.

The pineal NAT activity was determined radioenzymatically according to Deguchi and Axelrod (1972) as modified by Parfitt *et al.* (1975). The concentration of melatonin was assessed radioimmunologically according to Charron *et al.* (1991), the concentration of dopamine (DA), norepinephrine (NE) and epinephrine (E) radioenzymatically according to Johnson *et al.* (1980) using CATECHOLA tests (Institute of Radioecology and Applied Nuclear Technique, Prague, Czech Republic). Corticosterone levels were determined fluorometrically according to Guillemin *et al.* (1958). Each group consisted of 6 rats, and results were evaluated using the non-paired t-test.

Results

Pineal gland

The NAT activity in irradiated rats did not differ from the values of the control group. Irradiation decreased the concentration of melatonin at 30 and 60 min, but at 120 min this was not statistically significant (Fig. 1).

The DA and NE concentrations were significantly lower 30 and 120 min after exposure, however, at 60 min they almost did not differ from those in the control group. An increase in the E concentration at 30 min was followed by its decrease 60 and 120 min after exposure, in comparison with the controls (Fig. 2). The weight of the pineals in irradiated and control rats were approximately the same.

Serum

The level of serum melatonin in irradiated rats did not substantially differ from that in the control group. Irradiation nonsignificantly enhanced the concentration of corticosterone at 30 min, but this difference was later significant (Fig. 3).

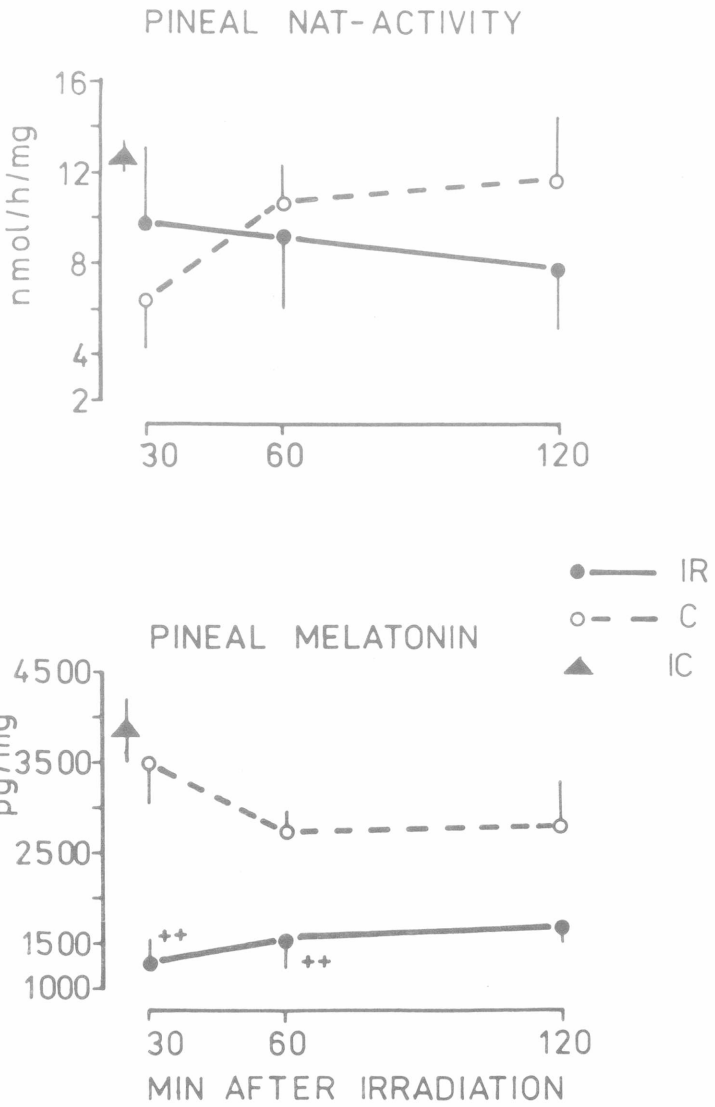

Fig. 1
Pineal N-acetyltransferase (NAT) activity and pineal melatonin concentration in irradiated (IR) and sham-irradiated (C) rats after exposure to a dose 14.35 Gy of gamma rays. Irradiated and sham-irradiated rats were analyzed in darkness. Triangles represent values of intact non-manipulated rats (IC). Results are expressed as means ± S.E.M., N = 6, ($^{+}$ $P<0.05$ and $^{++}$ $P<0.01$, IR vs C).

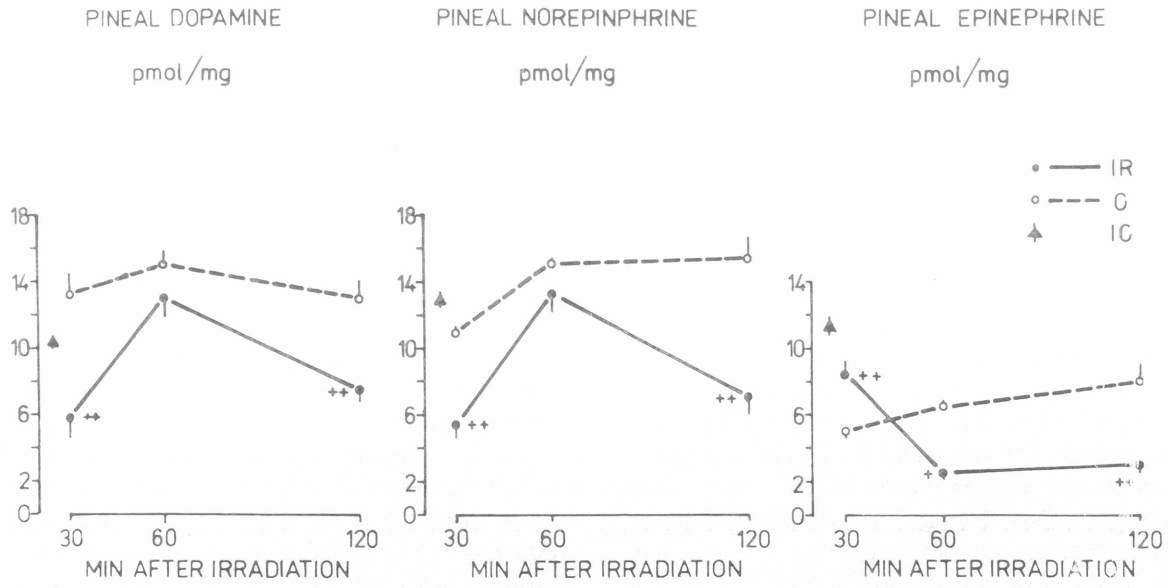

Fig. 2
Pineal dopamine, norepinephrine and epinephrine concentration in irradiated and sham-irradiated rats. Other details as in Fig. 1.

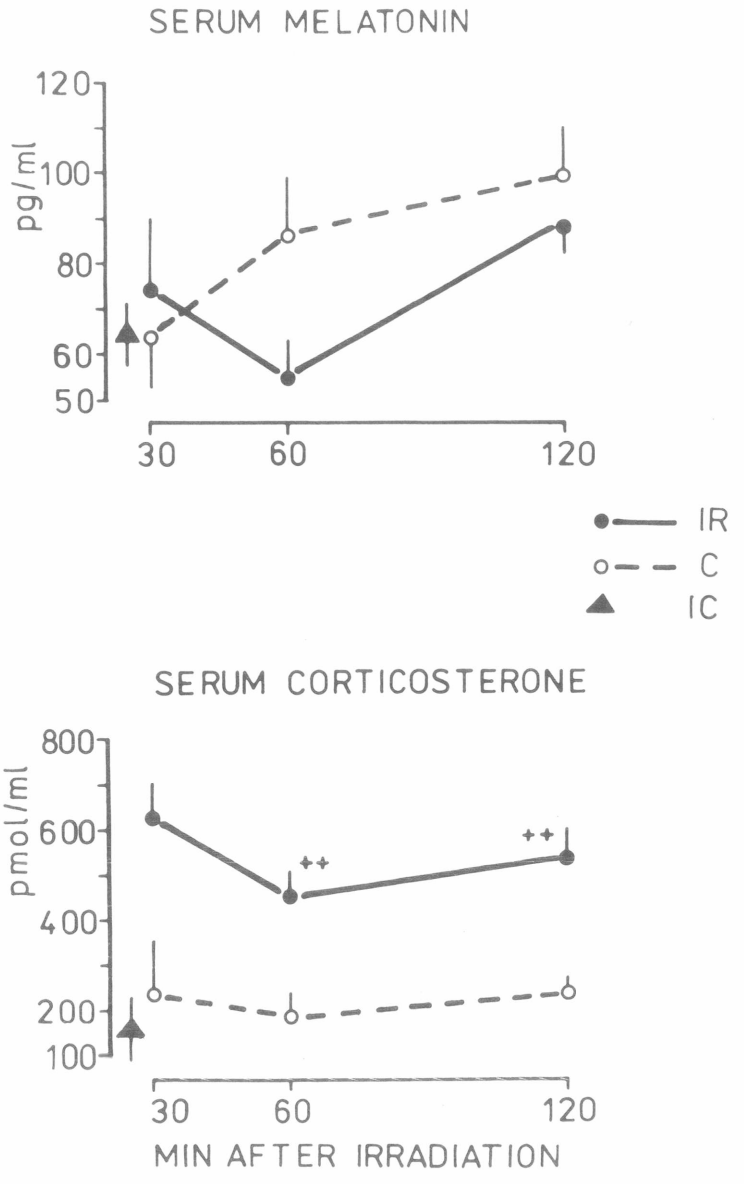

Fig. 3
The concentration of serum melatonin and corticosterone in irradiated and sham-irradiated rats. Other details as in Fig. 1.

Discussion

Melatonin is produced from the amino acid tryptophan *via* serotonin (5-hydroxytryptamine). The enzyme NAT converts serotonin to N-acetylserotonin and this is metabolized to melatonin (N-acetyl-5-methoxytryptamine) in the presence of the enzyme HIOMT.

Single whole-body irradiation of rats with a dose of 14.35 Gy diminished pineal melatonin concentration 30 to 120 min after exposure, but it did not change NAT activity. In a similar experimental model, an increase of NAT activity was recorded between 2300 to 2400 h in the pineal gland of rats on the 3rd and 4th days after the exposure (Kassayová *et al.* 1993a), which was accompanied by an increase in serum melatonin concentration at the same time (unpublished results). This indicates that the synthesis of melatonin during the period of fully manifested radiation injury is enhanced. Local irradiation of the rat head with a dose of 14.35 Gy of gamma rays did not change either NAT activity or the pineal melatonin content 120 min after the exposure (decapitation was performed between 2300–2400 h). Twenty-four and 72 hours later, the levels of pineal and serum melatonin of irradiated animals were increased, without any changes in NAT activity; between day 5 and 10 the values of melatonin did not differ from those in the controls (Kassayová *et al.* 1993b). A relatively low dose, 350 R of X-rays, lowered the activity of HIOMT in the rat pineal gland one and 24 h after the exposure (Ellis *et al.* 1970). A decrease in serotonin concentration in the brain, spleen, blood and intestine was described in whole-body irradiated rats with various X-ray doses (Ershoff *et al.* 1962, Matsuoka *et al.* 1962). An increase in monoamine oxidase (MAO) activity in the brain induced by ionizing radiation (Pausescu *et al.* 1976, Pástorová and Arendarčík 1988) can enhance oxidative deamination of serotonin, a metabolic pathway producing 5-hydroxyindol acetic acid (HIAA) and 5-hydroxytryptophol. Palaic *et al.* (1964) observed a high concentration of 5-hydroxyindolyl compounds in

rat brains shortly after irradiation with 9 Gy of X-rays. Lerchl *et al.* (1991) described reduced NAT activity, lower melatonin levels and an increase of serotonin and HIAA in the pineal gland of rats exposed at night to induced electrical currents produced by magnetic field pulses. The effects of "eddy" currents on pineal indolamine metabolism is similar to the effect of a light signal applied in darkness, but the mechanisms still remain obscure (Reiter and Richardson, 1992). We assume that in our experiment the low melatonin concentration in the pineal gland shortly after irradiation could be a consequence of changes in pineal serotonin metabolism.

A decrease in DA and NE concentration in the pineal gland was recorded 30 and 120 min after exposure. Decreased NE levels in the hypothalamus and NE and E levels in the pineal gland were recorded in ewes 5 days after continuous irradiation with a daily dose of 0.5 Gy up to an accumulated dose of 2.5 Gy of gamma rays and then stimulated with serum gonadotropin (Pástorová and Arendarčík 1989). In our work, a decrease in pineal NE concentration at 30 and 120 min after irradiation was paralleled by the low concentration of pineal melatonin, the synthesis of which is regulated by the adrenergic system. We assume that the decrease of the concentration of NE and its precursor DA in the pineal gland 30 and 120 min after the exposure could be the result of radiation damage of adrenergic fibre function. Ionizing radiation increased the activity of degrading enzymes of catecholamines, namely MAO activity, as has been found in the whole brain of rabbits (Pausescu *et al.* 1976) and in the hypothalamus of ewes (Pástorová and Arendarčík 1988).

In our experiment, changing patterns of pineal E were recorded in the early period after exposure. The increase in E concentration immediately after exposure is probably the result of the stress effect of radiation. It could be explained by increased uptake of E from the circulation. Kvetňanský *et al.* (1979) noticed an increase of pineal E after immobilization of rats. Immobilization for 20–240 min raised the pineal E level without evident changes in NE and DA levels. Adrenalectomy and adrenodemedullation performed before immobilization did not alter pineal E levels of unstressed controls substantially, but it prevented its increase after immobilization. This indicates that an extraadrenal source of pineal E is involved under basal conditions (Saavedra 1980).

The relationship of glucocorticoids to pineal melatonin levels is not clear. An increased level of serum corticosterone in the early period after irradiation was recorded in our present, and also in previous experiments with local irradiation of the rat head (Kassayová *et al.* 1993b). In *in vitro* experiments, corticosterone and dexamethasone significantly decreased the norepinephrine- and cAMP-stimulated content of melatonin in the pineal gland and incubation medium, but they did not influence the basal values of this hormone (Fevre-Montange and Abou-Samra 1983).

Based upon our previous and present results (Kassayová *et al.* 1993a), it may be stated that the pineal response in whole-body lethally irradiated rats is two-phased. In the early phase, an isolated decrease in pineal melatonin occurs which is accompanied by a reduction in pineal catecholamines and an increase in serum corticosterone. Later, the signs of increased melatonin synthesis dominate in the phase of manifested radiation injury. The mechanisms of these changes remain unknown and will be studied in future experiments.

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References

BARFUSS D.W., 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.

BAYER A., MALÍNSKÝ J., BAYEROVÁ G.: Ultrastructure of the pineal gland of rat after X-ray irradiation. *Acta Universitatis Palackianae Olomucensis Facultatis Medicae* **60:** 63–66, 1970.

CHARRON G., SOULOUMIAC I., FOURNIER M.C., CANIVENC R.: Pineal rhythm in 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. *Anal. 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. I. The pineal gland and melatonin synthesis as a possible mediator of the response. *Life Sci.* **9:** 1011–1020, 1970.

ERSHOFF B.H., HELLMERS R., WELLS A.F.,: Effects of radioprotective agent on tissue serotonin levels in the irradiated rat. *Proc. Soc. Exp. Biol. Med.* **110:** 536–538, 1962.

FEVRE-MONTANGE M., ABOU-SAMRA A.B.: Glucocorticoids inhibit the in vitro melatonin production by rat pineal stimulated by norepinephrine (NE) or 8-bromocyclic AMP. *Neuroendocrinol. Lett.* **5:** 403, 1983.

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.

JOHNSON G.A., KUPIECKI R.M., BAKER C.A.: Single isotope derivate (radioenzymatic) methods in measurement of catecholamines. *Metabolism* **29:** 106–113, 1980.

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.

KVETŇANSKÝ R., KOPIN I.J., KLEIN D.C.: Stress increases pineal epinephrine. *Commun. Psychopharmacol.* **3:** 69–72, 1979.

LERCHL A., HONAKA K.O., REITER R.J.: Pineal gland "magnetosensitivity" to static magnetic fields is a consequence of induced electric currents (eddy currents). *J. Pineal Res.* **10:** 109–116, 1991.

MATSUOKA O., TSUCHIYA T., FURUKAWA Y.: The effect of X-irradiation on 5-hydroxytryptamine (serotonin) content in the small intestine. *J. Radiat. Res.* **3:** 104–108, 1962.

PALAIC D., DEANOVIC Z., SUPEK Z.: 5-hydroxyindolyl compounds in the rat brain after X-irradiation. *J. Neurochem.* **11:** 761–753, 1964.

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.

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.

PÁSTOROVÁ B., ARENDARČÍK J.: Effect of protracted exposure to gamma radiation and hormonal stimulation with serum gonadotropine on the contents of catecholamines in hypothalamus, epiphysis, and adrenal glands of ewes (in Slovak). *Vet. Med. (Praha)* **34:** 171–180, 1989.

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.

REITER R.J., RICHARDSON B.A.: Magnetic field effects on pineal indoleamine metabolism and possible biological consequences. *FASEB J.* **6:** 2283–2287, 1992

SAAVEDRA J.M.: Brain and pineal adrenaline: its level, synthesis and possible function in stress. In: *Catecholamines and Stress. Recent Advances.* E. USDIN, R. KVETŇANSKÝ, I.J.KOPIN (eds), Elsevier-North Holland, New York, 1980, pp. 37–46.

STEPANOVIC S.R., NIKOLIC J.V., VARAGIC V.M.: The effect of L-DOPA and benserazide on the amount of catecholamines in the rat heart atria, hypothalamus and corpus striatum of X-irradiated rats. In: *Catecholamines and Stress. Recent Advances.* E. USDIN, R. KVETŇANSKÝ, I.J. KOPIN (eds). Elsevier-North Holland, New York, 1980, pp. 137–140.

SUGDEN D.: Melatonin biosynthesis in the mammalian pineal gland. *Experientia* **45:** 922–932, 1989.

VARAGIC V., STEPANOVIC S., SVECENSKI N., HAJDUKOVIC N.: The effect of X-irradiation on the amount of catecholamines in heart atria and hypothalamus of the rabbit, and in brain and heart of the rat. *Int. J. Radiat. Biol.* **12:** 113–119, 1967.

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