Melatonin Synthesis in Rat Pineal Glands Inhibited by X-Irradiation[1]

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Recent investigations suggest that the pineal gland functions as a true endocrine organ by influencing the reproductive systems of the rat (1). Evidence has been presented to show that the pineal influences testicular function in male rats (2, 3). In this respect, exposure to constant light reportably diminishes the ability of pineal gland to synthesize melatonin, while constant darkness enhances this ability (4). It has also been shown that pinealectomy affects the female in a way similar to that associated with the exposure to constant light, but the two treatments are not additive (5). Histological changes in the pineal gland have been reported after X-irradiation (6), but no attempt has been made to evaluate pineal function after this treatment in terms of the gland's ability to synthesize melatonin. We now report an inhibitory effect of X-irradiation on the ability of the pineal gland to synthesize melatonin 24 hr after treatment.

Materials and Methods. In studying this phenomena, male rats (Holtzman strain) were maintained in a small colony with a southern exposure. Artificial lighting was used only during the daylight hours when the caretaker was caring for the animals. Feed and water were given *ad libitum* during the course of the investigation. When 12 weeks of age, 5 of the 10 animals were irradiated with 450 R of X-rays (Seifert 150 kVp X-ray machine) A 1-mm Al filter was used to filter out the soft X-rays. Twenty-four hr later the animals were sacrificed with ether anesthesia, and the pineal glands were rapidly removed and weighted on an analytical balance. The assay system used was that of Axelrod and Weissbach (8) modified as follows for thin-layer chromatography and individual organs: the pineal glands were homogenized in 0.1 ml of *N*-acetylscrotonin (20 mμmoles) in 0.5 *M* phosphate buffer, pH 7.9, in an all glass microhomogenizer (Kontes). The homogenates were transferred to 15-ml glass stoppered conical test tubes containing 0.1 ml of ^{14}C-methyl-5-adenosyl methionine (0.05 μCi, 55 mCi/mmole). Incubation of the samples was carried out in a constant temperature bath at 37° for 90 min. Twenty-five μg of carrier melatonin were added to the samples after the incubations were terminated by the addition of 8 ml of chloroform to the homogenates. After shaking the samples for 15 min, the emulsions were broken by centrifugation, and the chloroform supernatants containing the radioactive and carrier melatonin were transferred to clean conical test tubes. All solvents were rigorously purified and redistilled prior to use.

The chloroform was evaporated from the samples under a stream of dry nitrogen gas at 45°. The walls of the test tubes were rinsed down three times with chloroform and methanol (1:1) to concentrate the melatonin in the tips of the tubes. Finally, 3–4 drops of chloroform and methanol (1:1) were added to the tubes after the final drying. The residues were transferred to 3-cm strips of thin-layer chromatofilm (silica gel with fluorescent indicator, Eastman Kodak Co.). The chromatograms were developed in either a methanol–water (95:5) or an alkaline propanol–NH_3 (8:2) system using the ascending

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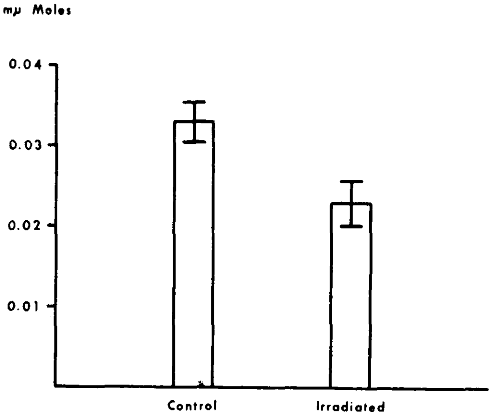

FIG. 1. Inhibition of melatonin synthesis by rat pineal glands 24 hr after whole-body radiation with 450 R of X rays.

technique. The strips were run through a 2 pi Geiger-Muller electronic autoscanner to locate the radioactive melatonin. Carrier melatonin was visualized by using an ultraviolet light scanner (254 mμ). The portions of the strips containing the radioactivity were cut out and placed in scintillation counting vials. After adding the scintillation cocktail, the vials were placed in a liquid scintillation counter (Packard Instr. Co.) and counted for 10 min. An average of three counts was obtained for each sample. The counting efficiency was 88% and external standardization was used to correct for quenching.

Discussion. The radioactive material isolated through this procedure was judged to be melatonin on the basis of the following criteria: (i) the isolation of the material with the specific extraction procedure for melatonin, (ii) the material migrated with authentic carrier melatonin in either the methanol–H_2O system or the alkaline system of propanol–NH_3, and (iii) no radioactive material was observed to migrate with the carrier melatonin when tissues other than the pineal were assayed with this sytem.

Statistical analysis of these data (Fig. 1) showed that the two means differ significantly ($p > 0.001$). These data show that whole-body irradiation does inhibit melatonin synthesis *in vitro* by decreasing the hydroxyindole-*O*-methyl transferase activity. In this respect, it is interesting to note that melatonin inhibits androgen synthesis *in vitro* (8) and that androgen synthesis is increased 24 hr after radiation (9, 10). These data would suggest a possible role for the pineal gland in regulating androgen synthesis immediately after radiation by removing an inhibition normally presented by melatonin. For a further discussion of the implications of melatonin synthesis as it relates to steroid synthesis and testicular function after exposure of rats to X-irradiation, the reader is referred to a recent review of this subject (11).

Summary. Melatonin synthesis was measured in individual male, rat pineal glands *in vitro*. When compared with control animals 24 hr after treatment, rats irradiated with 450 R of X rays demonstrated a striking decrease in the activity of the melatonin-synthesizing enzyme hydroxyindole-*O*-methyl transferase in the pineal glands.

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