BLEACHING ACTION OF EXTRACT OF THE MUCOSA AND SUBMUCOSA OF THE HUMAN APPENDIX ON MELANOPHORES OF THE FROG SKIN

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The action of a supernatant obtained after extraction of a homogenate of the mucosa and submucosa of the human appendix with 95% acetone on the melanophores of the frog skin was investigated. A bleaching action was found, indicating that the homogenate contained melatonin. Considering the high content of the melatonin precursor, 5-hydroxytryptamine (serotonin), in the enterochromaffin cells, the possibility of melatonin biosynthesis in these cells during serotonin metabolism can be postulated.

KEY WORDS: enterochromaffin cells; melatonin biosynthesis; melatonin precursor – 5-hydroxytryptamine (serotonin).

McCord and Allen [4] described bleaching of the head of tadpoles after they had been fed on plant food mixed with bovine pineal gland. This bleaching lasted about 3 h and was visible with the naked eye. Later, Lerner et al. [3] isolated a hitherto unknown substance, 5-methyoxy-N-acetyltryptamine, from the bovine pineal gland, a derivative of 5-hydroxytryptamine (serotonin) with an exceptionally strong bleaching action on melanophores of the frog skin, on account of which it was called melatonin.

Melatonin is now regarded as a specific hormone of the pineal gland, and great importance is attached to it in the regulation of vital processes [1, 2].

The main site of biosynthesis and metabolism of the melatonin precursor, 5-hydroxytryptamine, in the body is now known to be the enterochromaffin (Ec) cells of the gastrointestinal tract. With this in mind it was postulated that melatonin is formed in the Ec cells during metabolism of serotonin.

EXPERIMENTAL METHOD

Human appendices removed at appendectomy were kept in liquid nitrogen for 12 h. On a freezing microtome with measured carbon dioxide feed to the knife, sections 10-15μ in thickness were cut through the mucosa and submucosa. The sections were homogenized with 3 volumes of isotonic sodium chloride solution. Extraction of the serotonin derivatives was carried out in the cold with 50 ml of 95% acetone solution. The supernatant was washed twice and used in the experiment.

The frog skin was first moistened for 1 h in cold Ringer's solution and stretched out on a plastic frame. The action of the supernatant, of 95% acetone, and of 10% caffeine on the melanophores was investigated, after which the darkened skin was incubated in the supernatant for 15 or 30 min, 1-5 h, and 1-5 days. The results were read on the same area of skin (50-60 melanophores per field of vision under low power) with a magnification of 56 ×, 600 ×, and examination with the naked eye.

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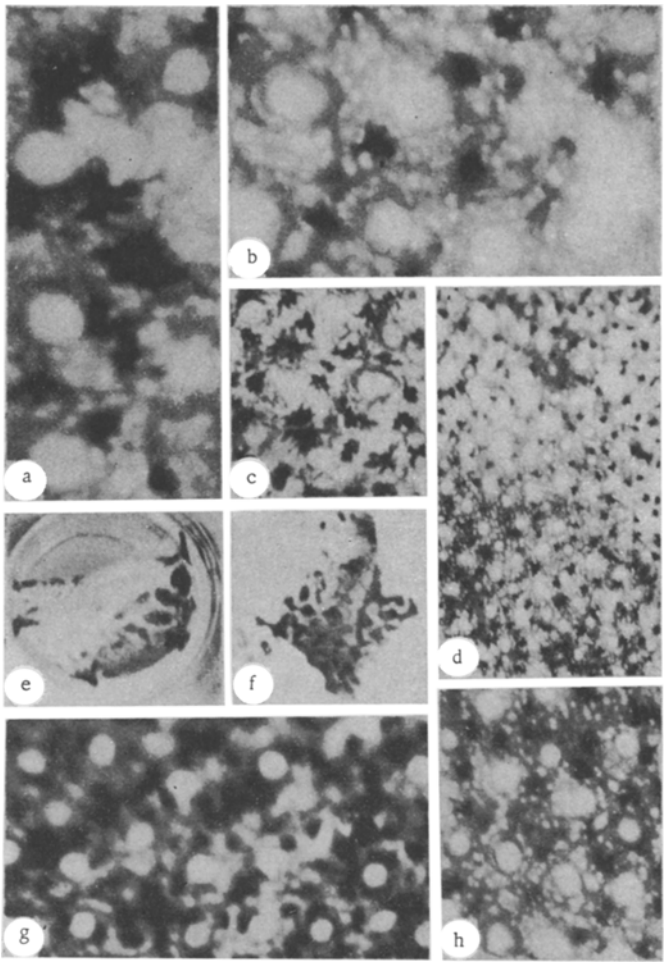

Fig. 1. Bleaching action of melatonin of Ec cells of the human appendix on melanophores of the frog skin: a) normal melanophores of the frog's skin; b) aggregation of pigment in perinuclear zone of melanophores after incubation of frog skin for 20 min in the test supernatant; c) melanophores of frog skin before treatment with test supernatant; d) decreased density of melanophores after incubation of frog skin for 1 h in supernatant; e) frog skin incubation for 5 h in supernatant: outlines of pigment grains are obliterated; f) normal frog skin; g) darkening of frog skin after incubation for 1 h in 10% caffeine; h) bleaching of frog skin, darkened after exposure to caffeine, 1.5 h after incubation in test supernatant. a, b) 600 ×; c, d, g, h) 56 ×; e, f) 3 × (magnifying glass).

EXPERIMENTAL RESULTS

From 15 to 20 min after incubation of the frog's skin in the supernatant distinct aggegation of pigment granules in the central part of the melanophores around the pale nucleus could be seen under high power. The processes of the melanophores were poorly outlined and appeared to be "fragmented" compared with the control (Fig. 1a, b).

Under low power 1 h after incubation, bleaching of the skin could be seen in the field of vision. The melanophores had become smaller because of the escape of pigment grains into the zone around the nucleus, and the distance between the individual cells had increased, giving the effect of a reduction in the number of melanophores per unit area and of bleaching of the skin (Fig. 1c, d).

By the end of the 5th hour the bleaching was visible with the naked eye: the pigment grains were less clearly visible and their outlines were blurred (Fig. 1e, f). On further incubation of the frog skin in the supernatant at 4°C the bleaching effect increased until the 5th day.

To rule out the possibility of obtaining positive results on account of the action of the extracting substance, the frog skin was incubated in 95% acetone for the same period of time. After incubation for 30 min the skin became hard and wrinkled, dark brown in color, and on microscopic examination the individual melanophores were almost indistinguishable. A 10% solution of caffeine caused definite darkening of the skin 1 h after incubation because of large aggregations of melanophores (Fig. 1g), and after 2-4 h this could be observed with the naked eye.

After washing the darkened skin in cold Ringer's solution followed by incubation in the supernatant, aggregation of the pigment grains from the processes of the cells began 1-1.5 h later. It was less distinct than after exposure to the supernatant without preliminary incubation of the frog skin in 10% caffeine (Fig. 1 h).

These results are evidence of the presence of melatonin in a homogenate of the mucosa and submucosa of the human appendix, for the effect described is specific for this hormone and it was demonstrated previously only in pineal extracts [1, 5].

Since the Ec cells are the principal site for the biosynthesis and metabolism of serotonin, the direct precursor of melatonin, there is reason to ascribe this action of the homogenate resembling the effect of melatonin to the Ec cells.

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