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IMMUNOHISTOCHEMICAL DETECTION OF THE LOCALIZATION OF MELATONIN AND N-ACETYLSEROTONIN IN ENTEROCHROMAFFIN CELLS

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Melatonin synthesis was identified by an immunohistochemical method with specific antisera against melatonin and N-acetylserotonin in the enterochromaffin cells of the gastrointestinal tract. It is considered that enterochromaffin cells, together with the pinealocytes of the pineal gland and other cells synthesizing melatonin in the retina and cerebellum, form a group of melatonin-producing cells with an important role in the maintenance of homeostasis.

KEY WORDS: *Melatonin; enterochromaffin cells; specific antisera.*

The important role of melatonin in the regulation of metabolic processes has recently been established [4, 7, 8].

Until recently the pineal gland has been considered to be the only organ synthesizing this hormone [9, 14], although it was not quite clearly understood how the relatively small quantity of melatonin produced in the pineal could maintain the course of metabolism at a high enough level.

Recent investigations have questioned the previous view of the leading role of the pineal in melatonin production [10, 11].

Since serotonin is essential for melatonin synthesis [8], the suggestion has been made that melatonin can be formed in the enterochromaffin cells of the gastrointestinal tract

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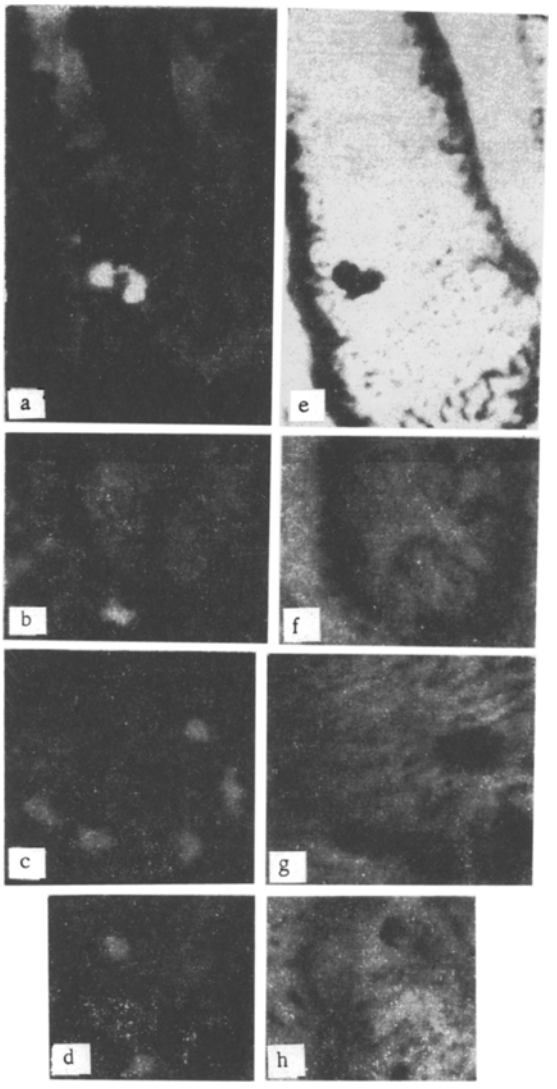

Fig. 1. Detection of enterochromaffin cells in crypts of mucous membrane of human appendix (a, b, c, e, f, g) and rabbit small intestine (d, h). Sections treated with: a) antiserum against melatonin, 900×; b) antiserum against N-acetylserotonin, 600×; c) antiserum against melatonin, 900×; d) antiserum against melatonin, 600×; e-h) Masson's N-argentaffin reaction, 900× (e, g) and 600× (f, h).

[1, 2], in which 90-95% of all endogenous serotonin is synthesized [13]. Subsequent investigations have shown that melatonin and its main presursors are present in extracts from the mucous membranes of the human appendix and the rabbit gastrointestinal tract, in which there are numerous enterochromaffin cells [3, 6]. Evidence of a link between melatonin and enterochromaffin cells is given by the fact that they contain serotonin [12] and by the clear correlation between changes in the number of enterochromaffin cells in the mucous membranes of appendices from which extracts were prepared and the time of development of the decolorizing action, characteristic of melatonin, on the skin melanophores of frogs after incubation in test extracts [5, 6].

The object of this investigation was the immunohistochemical determination of melatonin and its immediate precursor, N-acetylserotonin, in enterochromaffin cells.

EXPERIMENTAL METHOD

Serial cryostat sections 10 μ in thickenss were cut from appendices removed during appendectomy and from the small intestine of rabbits. The sections were mounted in the cryostat chamber on slides and fixed with cold acetone for 2-3 sec. The sections were then dried in air at room temperature for 20 min and incubated in a few drops of specific antiserum against melatonin or N-acetylserotonin for 1 h. Before use, the required quantity of each antiserum was treated with bovine serum albumin in the ratio of 1 g to 1 ml antiserum and the mixture was diluted with isotonic buffered sodium chloride solution (pH 7.2, 1:4). After incubation of the sections in one or other antiserum they were washed carefully in several portions of the same buffer solution and dried at room temperature, after which they were treated for 30 min with an isothiocyanate-labeled donkey luminescent serum against rabbit globulins (from the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR). After washing in the same buffer the sections were transferred to buffered glycerol solution (1 part buffer to 9 parts glycerol) and examined in the ML-24 luminescence microscope (FS 1-2 and SZD 7-2 filters). Identical serial sections were stained by Masson's argentaffin method to detect enterochromaffin cells [15] and also with hematoxylin and eosin.

Immune antisera were prepared by the method of Grote and Brown (1974). Hapten (50 mg) was dissolved in 2 ml distilled water and added to a solution containing 150 mg bovine serum albumin in 3 ml water. Next, 2 ml of 3 M sodium acetate solution and 2 ml 7.5% formaldehyde were added (pH of the mixture 6.8). Dialysis was carried out in running water for 3 days. After centrifugation of the solution (15,000 rpm, 40 min) the quantity of attached hapten was determined spectrophotometrically, from the ratio between absorption in the ultraviolet at 280 and 300 nm. The conjugates thus obtained contained 12-18 moles hapten per mole protein.

Three injections, each of 0.5 mg antigen in Freund's incomplete adjuvant, were given subcutaneously to the rabbits in the dorsal region. Reimmunization was carried out 1 month later, when 0.5 ml of the conjugate was injected intravenously. Serum was taken on the 10th day after reimmunization and antibodies were determined by the immunodiffusion method.

To determine the specificity of action of the antisera some sections were incubated in antisera against melatonin and N-acetylserotonin without preliminary treatment with bovine albumin, whereas other sections were treated with donkey luminescent serum against rabbit globulins only without previous incubation in antisera against melatonin and N-acetylserotonin.

EXPERIMENTAL RESULTS

On treatment of the sections with specific antisera against melatonin and N-acetylserotonin intense yellow fluorescence was observed in the crypts of the human appendix and the rabbit small intestine. Both single cells (Fig. 1a, b) and groups of cells (Fig. 1c, d) were luminescent and, by their arrangement and number, they were evidently identical with enterochromaffin cells, giving a positive argentaffin reaction when stained by Masson's method (Fig. 1e-h).

A nonspecific, weak, and total yellow luminescence was observed in the control sections.

The discovery of specific luminescence of the enterochromaffin cells in sections treated with antiserum against melatonin is conclusive evidence that melatonin is present in the intestinal enterochromaffin cells. The presence of N-acetylserotonin, the direct precursor of melatonin, which also gives a positive immunohistochemical reaction with its specific antiserum, in the same cells is evidence of active synthesis of melatonin in the enterochromaffin cells. Considering the large number of these cells in the gastrointestinal tract, many more than the pinealocytes in the pineal gland or cells responsible for the synthesis of this hormone in the retina and cerebellum, it must be admitted that not only is the pineal gland not the only organ producing melatonin, but it is likewise not the major producer. Identification of enterochromaffin cells as melatonin producers and the presence of other melatonin-producing cells in the body imply the existence of a group of cells whose specific function is to produce melatonin, which gives them an important physiological role.

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