

## IMMUNOHISTOCHEMICAL DETECTION OF CELLS CONTAINING MELATONIN AND N-ACETYL-SEROTONIN

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UDC 612.826.33.018-087.4

The presence of cells containing N-acetylindolylalkylamines in the acini of the pancreas, at the boundary between the adrenal cortex and medulla, beneath the capsule of the liver, and in the wall of blood vessels was demonstrated by an immunohistochemical method using specific antisera against melatonin and N-acetylserotonin. Further investigations are necessary for the morphological identification of the type of these cells.

KEY WORDS: melatonin; endocrine cells; specific antisera

Considerable attention is being paid at the present time to the study of substances connected with the regulation of physiological functions. These include melatonin, which is formed in the body during serotonin metabolism. Besides in the pineal gland [10], melatonin is also found in the retina [6], cerebellum [4], enterochromaffin cells of the gastrointestinal tract [2, 3], and the harderian glands of rats [5]. Cells of these various organs containing melatonin are largely identical in their morphological and histochemical properties. The enterochromaffin cells (Kulchitsky's cells) are typical representatives of them. Endocrine cells similar to them, giving all the positive reactions characteristic of the melatonin-containing cells described above, have recently been found in the acini of the pancreas [1], beneath the capsule and in the sinusoidal tissue of the liver [11], in the cortical and juxtaglomerular zone of the kidneys [9], and on the boundary between the adrenal cortex and medulla [7]. However, their function has not yet been explained.

The object of this investigation was to attempt to identify the possible presence of melatonin and of its immediate precursor, N-acetylserotonin, in the regions of concentration of these cells in certain organs.

### EXPERIMENTAL METHOD

Pieces of the pancreas, adrenals, kidneys, and liver taken from mongrel dogs served as the material for study. Cryostat sections were mounted on slides and fixed for 2-3 sec with cold acetone. After the sections had been dried in the air at room temperature they were incubated in a few drops of specific antisera against melatonin or N-acetylserotonin for 1 h, after which they were treated with a donkey luminescent serum against rabbit globulins, labeled with fluorescein isothiocyanate [3]. The method of obtaining the immune antisera against melatonin and N-acetylserotonin [8] was described previously [3]. Immunological specificity was confirmed by the following control experiments: Sections were incubated in the donkey luminescent serum only, without preliminary incubation in the specific antisera, and parallel tests were carried out on sections from organs whose cells do not contain melatonin or N-acetylserotonin (myocardium, uterus) and sections of the human appendix, in whose enterochromaffin cells melatonin was identified previously by the same method [3].

### EXPERIMENTAL RESULTS

On incubation of cryostat sections of the various organs in the specific antisera against melatonin and N-acetylserotonin, bright yellow luminescence of individual cells and cell clusters was found in the acini of the pancreas (Fig. 1a), at the boundary between the adrenal cortex and medulla (Fig. 1b), beneath the capsule of the liver (Fig. 1c), and in the renal cortex (Fig. 1d). After treatment of the sections with specific antiserum against melatonin, luminescence also was found in the wall of a blood vessel in the kidney.

Sections from the myocardium and uterus, treated with specific antisera against melatonin and N-acetylserotonin, did not give luminescence. Meanwhile, luminescence was observed in the enterochromaffin cells.

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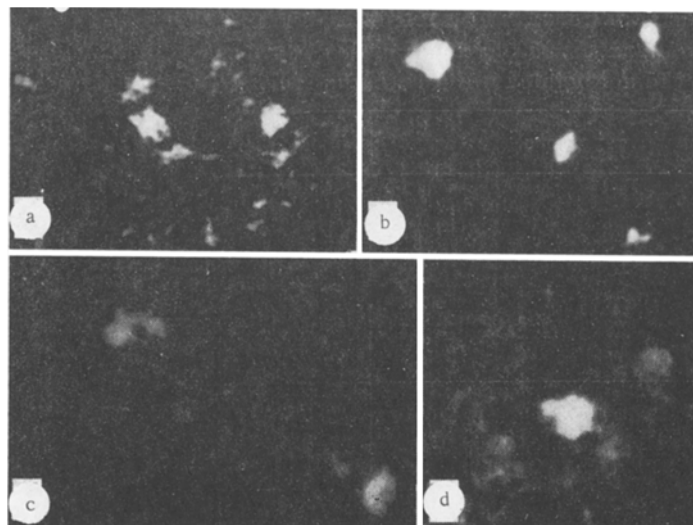


Fig. 1. Endocrine cells of various organs containing melatonin: a) cells in acini of pancreas (600  $\times$ ); b) cells in adrenal cortex on boundary with medulla (900  $\times$ ); c) cells located immediately beneath the capsule of the liver (900  $\times$ ); d) cells in renal cortex (900  $\times$ ). a, b, c, d) Sections treated with antiserum against melatonin.

The results are evidence that cells containing N-acetyldolylalkylamines (melatonin and N-acetylserotonin) are present in the pancreas, liver, kidneys, adrenals, and walls of blood vessels in areas where the presence of chromaffin cells was demonstrated previously by other workers. Since the antisera used can give cross-reactions [8], it cannot be stated categorically that melatonin can be synthesized in these structures, but the fact that they contain N-acetyldolylalkylamines was clearly demonstrated. Further investigations are necessary for the morphological identification of the type of these cells.

The authors are grateful to Candidate of Medical Sciences Z. G. Kadagidze and to A. V. Sokolov for help with the preparation of the antisera.

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