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AUTORADIOGRAPHIC AND IMMUNOHISTOCHEMICAL ANALYSIS OF ^{3}H-MELATONIN DISTRIBUTION IN ENDOCRINE AND NONENDOCRINE ORGANS

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In recent years particular attention has been paid by research workers to malatonin (5-methoxy-N-acetyltryptamine), a hormone of the APUD system [4]. It has been shown that the pineal gland is not the only source of melatonin formation in the body. The use of biological methods, radioimmunoassay, and thin-layer chromatography has shown that after removal of the pineal gland melatonin continues to appear in the plasma and uring of experimental animals [16]. The 24-hourly excretion of melatonin in rats after pinealectomy is about 20% of the control value. The use of methods of immunohistochemistry, with highly specific antibodies to melatonin, has established the identity of the melatonin-containing cells in certain regions of the brain, the retina, the gastrointestinal tract, and other organs [3, 4, 6, 7, 8]. Melatonin has a broad spectrum of physiological action [1, 4, 10, 13] and it is regarded nowadays as a universal regulator of biological rhythms. We know that exogenous methoxyindoles have a short half-life and that they are metabolized to a very considerable degree in the liver [11, 15, 17, 18]. Unmetabolized melatonin binds with plasma proteins, with albumin perhaps [11]. The physiological importance of binding of circulating melatonin with proteins has not yet been established. An hour after injection of tritium-labeled melatonin the concentration of the radioactive preparation in the pineal gland is 40 times higher, whereas in endocrine organs, peripheral nerves, and sympathetic ganglia, it is 3-5 times higher than in blood plasma [18]. Accumulation of radioactive label has been observed in the liver, kidneys, small intestine, and adrenals. Data indicating that tissues containg endogenous melatonin have high affinity for injected exogenous melatonin are of undoubted interest [9, 12, 18]. These facts are evidence of the urgent importance of the study of melatonin and the clarification of some problems connected with the transport, storage, and pathways of utilization of exogenous melatonin in the body.

This paper gives details of an autoradiographic and immunohistochemical investigation of the distribution of ^{3}H-melatonin in the early period after its administration, in certain endocrine and nonendocrine organs.

EXPERIMENTAL METHOD

Experiments were carried out on male BDF_1 mice. ^{3}H-Melatonin was injected intraperitoneally in a dose of 185 kBq/g. The animals were killed by decapitation 5 min and 1 and 3 h after injection of the labeled compound. The adrenals, and pieces of the duodenum, pancreas, and spleen were fixed in buffered Bouin's fluid and embedded in paraffin wax. Material for autoradiography was prepared and photographed by the standard method, using type "M" photographic emulsion. Melanotonin-containing were identified by the indirect immuno-

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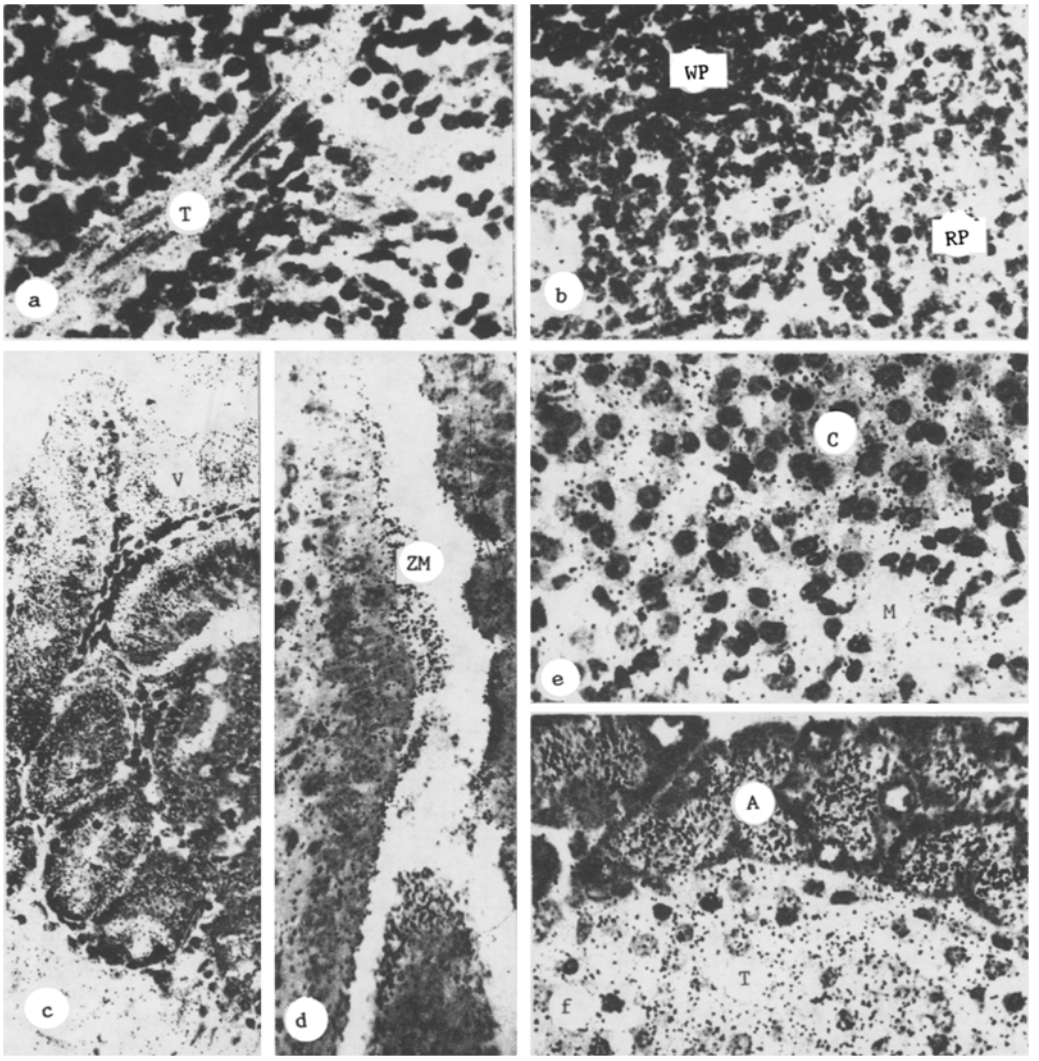

Fig. 1. Distribution of label in endocrine and nonendocrine organs after injection of ^{3}H-melatonin: a, b) spleen. Accumulation of label in connective tissue after 5 min (a) and in cells of red and white pulp after 1 h (b). T) Trabecula of connective tissue, RP) red pulp, WP) white pulp. 540 ×; c, d) Duodenum. Concentration of label in villi after 1 h (c) and in zone of mucous deposits after 3 h (d); V) villi, ZM) zone of mucous deposits. Magnification: c) 270, d) 540 ×; e) adrenal. Concentration of label in cortical cells 5 min after injection of ^{3}H-melatonin, C) cortex, M) medulla. 540 ×; f) Pancreas. Grains of silver located predominantly in acinar cells after 1 h. A) Acini, I) islets of Langerhans. 540 ×.

peroxidase method, using rabbit antiserum to melatonin and goat antibodies to rabbit immunoglobulin, conjugated with peroxidase ("Calbiochem"). The sequence of immunohistochemical treatment was the same as in the method in [2]. Serial sections were stained with hematoxylin and eosin, with toluidine blue to detect mast cells, by Masson's argentaffin method, and by Grimelius' argyrophilic method to identify endocrine cells. Intact mice served as the control. The ^{3}H-melatonin was obtained by acetylation of 5-methoxytryptamine with acetic anhydride, labeled with tritium. The specific radioactivity of the melatonin thus obtained was 1.25 GBq/mmole. Th epurity of the ^{3}H-melatonin was verified by thin-layer chromatograhy. Chromatographic analysis demonstratd the identify of the melatonin preparation obtained and a standard specimen of melatonin; RF of the two preparations was identical, namely 0.685, whereas for the original 5-methoxytryptamine RF = 0.33. Antiserum to melatonin was obtained in the Laboratory of Clinical Immunology, All-Union Ocologic Scientific Center, Academy of medical Sciences of the USSR, by the method in [14].

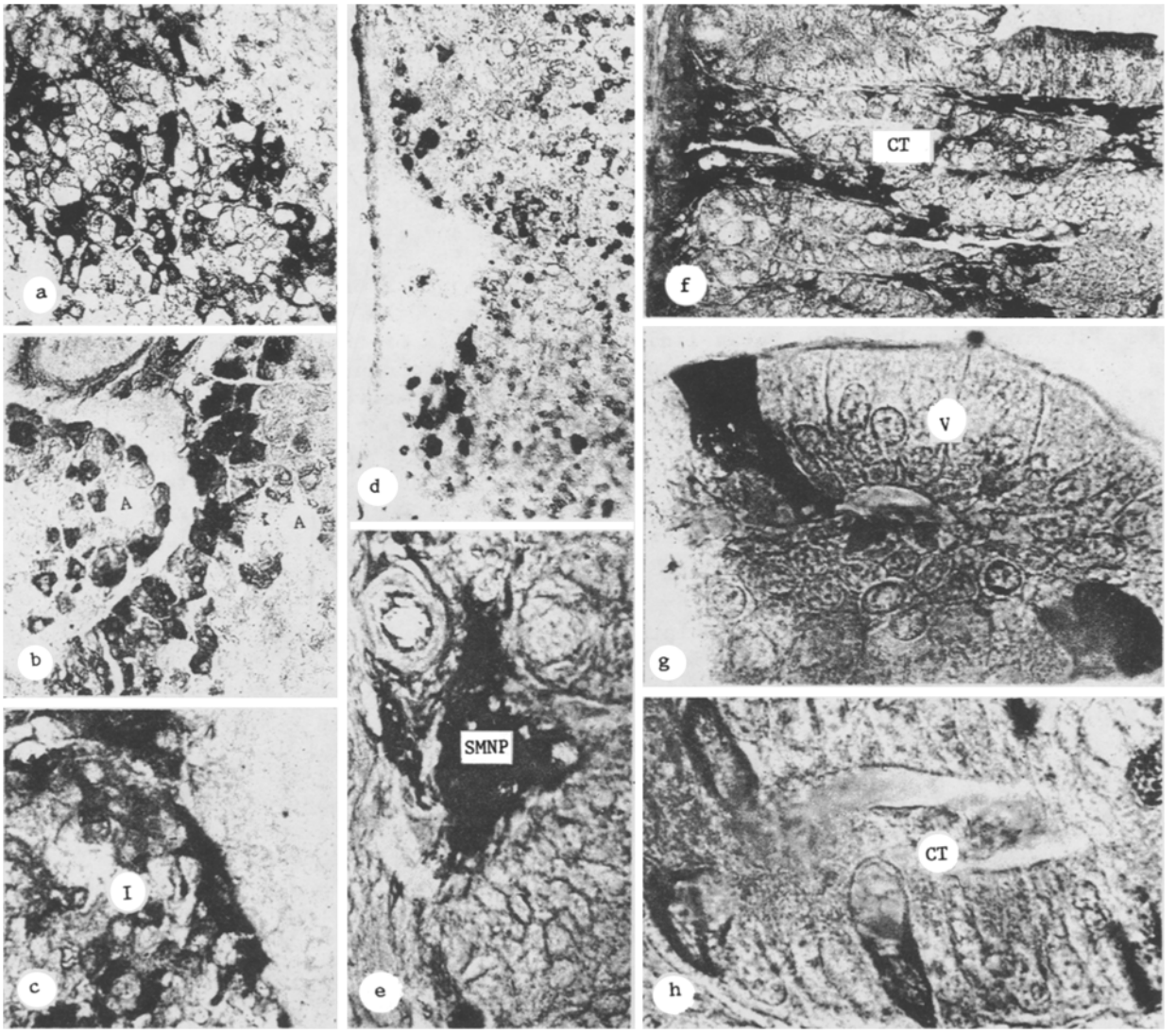

Fig. 2. Melatonin-immunoreactive cells in different organs after injection of ^{3}H-melatonin. a) Adrenal. Positive reaction of cells in medulla 5 min after injection of melatonin. 240 ×; b, c) Pancreas. Accumulation of melatonin in acinar cells after 1 h (b) and in endocrine cells of islet after 3 h; A) acini, I) islets of Langerhans. Magnification: b) 200, c) 400 ×; d) spleen. Positive reaction of cells after 1 h; 240 ×; e-h) duodenum. (e, f) 5 Min, G, h) 3 h after injection of melatonin; SMNP) submucous nerve plexus, CT) connective tissue (arrows indicate mast cells), V) villus, Cr) crypt. Magnification: e) 600, f) 300 g, h) 1200 ×.

EXPERIMENTAL RESULTS

Autoradiographs were found in all the organs studied 5 min after injection of the labeled preparation, with their predominant localization in the stroma (Fig. 1a). The concentration of label in the spleen increased appreciably after 1 and 3 h (Fig. 1b). However, the label was found most intensively in the adrenals, pancreas, intestine. In the duodenal mucosa autoradiographs were distributed relatively diffusely. Some degree of selectively was recorded above individual stromal cells after 5 min, and accumulation of the label in the epitheliocytes of the villi also was found 1 h (Fig. 1c), and in the zone of mucous deposits in 3 h (Fig. 1d) after injection of the preparation. In the adrenals and pancreas, the nonhomogeneity of distribution of the label could be clearly identified: in the adrenals the intensity of labeling was higher in cells of the cortex (Fig. 1e), in the pancreas in the acinar cells (Fig. 1f).

In intact animals, within the range of dilutions of the first layer of antibodies used in the investigation, cells giving a positive reaction with antiserum to melatonin were found only in the duodenum. In the intestinal mucosa these cells were distributed among the epitheliocytes of the crypts and villi and they corresponded in their distribution to EC-cells, giving a positive argentaffin reaction.

After injection of ^{3}H-melatonin the histologic picture of distribution of immunoreactive cells appeared as follows (Fig. 2). In the adrenals, melatonin-containing cells were located in the medulla (Fig. 2a). In the pancreas after 5 min and 1 h, individual acinar cells gave a reaction for melatonin (Fig. 2b). After 3 h, cells with a positive reaction were found in the islets of Langerhans (Fig. 2c). In serial sections, a positive argyrophilic Grimelius' reaction was characteristic of the immunoreactive cells of the islets. In the spleen, endogenous melatonin accumulated in cells lining the sinusoids of the red pulp (Fig. 2d). The distinctive histotopography of distribution of the cells accumulating melatonin was observed in the duodenum. After only 5 min, nodes of the submucous nerve plexuses (Fig. 2e) and cells of the connective-tissue lamina of the mucosa (Fig. 2f) gave an intensely positive reaction. By the reaction of metachromasia, individual cells in the stroma were identified as mast cells. The intensely strong reaction of the mast cells also persisted 1 h after injection of melatonin. However, after 3 h most of the mast cells no longer gave a reaction for melatonin. At the same time there was a sharp increase in the number of cells containing melatonin in the crypts and villi (Fig. 2g, h).

Analysis of the data shows disparity between the distribution of radioactive label and the histotopographic distribution of melatonin-containing cells in all the organs studied. This was evidently due to differences in the recording parameters of the methods used. Immunohistochemical methods revealed exclusively unchanged melatonin, whereas autoradiography can record not only ^{3}H-melatonin, but also its labeled metabolic products. Incidentally, in preparations treated by the immunoperoxidase method, followed by application of photographic emulsion, no significant increase in the amount of label could be found above cells with a strongly immunopositive reaction. This fact may indicate that the radioactive concentration of unmetabolized ^{3}H-melatonin accumulating in individual cells not only did not exceed, but may even have been much lower than the concentration of its labeled metabolic products in these cells. Comparison of the autoradiographic and immunohistochemical data indicates that products of melatonin metabolism very quickly spread throughout the body and are taken up most rapidly by cells with a high level of biosynthesis, cells of the adrenal cortex, the acinar cells of the pancreas, and epitheliocytes of the duodenal villi.

In intact animals, immunohistochemical investigations did not reveal melatonin in organs such as the liver, spleen, and pancreas [7]. There is evidence of the concentration of exogenous melatonin in the gastrointestinal tract and salivary glands 1 day after its administration [8, 9]. The accumulation of exogenous melatonin which we observed both in individual endocrine cells and in nonendocrine cells of endocrine and nonendocrine organs, is therefore particularly interesting.

Comparison of the results of the autoradiographic and immunohistochemical investigation thus demonstrates that exogenous ^{3}H-melatonin is rapidly metabolized, and its metabolic products are spread throughout the body. In the early stages after administration, exogenous melatonin can accumulate in individual endocrine and nonendocrine cells of different organs, and have a direct regulatory effect on their function.

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USE OF A LECTIN-HISTOCHEMICAL METHOD TO CHARACTERIZE GLYCOSYLATION OF BIOPOLYMERS IN DUODENAL GLAND CELLS

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The use of lectins as a new class of histochemical reagents has led to considerable progress in recent years in our understanding of the structure and function of complex carbohydrates and carbohydrate-containing biopolymers of cells and tissues [4, 6, 8, 11]. With the aid of lectins not only can specific glycoconjugates and the cells accumulating them be identified, but they can also act as quite sensitive molecular probes, which can be used to study the redistribution of carbohydrate-containing biopolymers under physiological and pathological conditions [4]. In the present investigation an attempt was made to utilize lectins for the morphologic analysis of certain stages of glycosylation of carbohydrate-containing biopolymers in duodenal gland cells.

EXPERIMENTAL METHOD

Samples of duodenum from sheep and cows were studied. The choice of animals was determined by previous data showing that the duodenal glands of herbivorous mammals have the broadest spectrum of lectin receptors [14]. Pieces of duodenum from healthy mature animals were excised from the proximal third of the duodenum, not more than 30 min after death. Since the functional state of the glands, connected with digestive processes, affects the results of histochemical detection of carbohydrates in glandulocytes [1], material for investigation was taken only from animals with no chyme in their stomach. Histologic specimens were fixed in 10% neutral formalin and embedded in paraffin wax.

Serial sections 7 μ thick were treated with the following lectins: concanavalin A (con A), lentil (LCA) – both specific for αD-mannose, peanut (PNA), caster oil (RCA) – both specific for βD-galactose, soy (SBA) – specific for N-acetyl-D-galactosamine, wheat germ agglutinin (WGA) – specific for N-acetyl-D-glucosamine and N-acetylneuraminic acid, Laburnum anagyroides (LAA) – specific for αL-fucose [4]. The corresponding glycopolymers were detected by means of lectins labeled with horseradish peroxidase, followed by visualization in a system of diaminobenzidine – H_2O_2 [3]. Conjugates of lectins with peroxidase, and also purified peroxidase, for indirect detection of con A receptors were obtained at the L'vov branch of the A. V. Palladin Institute of Biochemistry, Academy of Sciences of the Ukrainian SSR. The cytotopography of the lectin receptors was analyzed separately in gland cells located at the periphery of the terminal portions and in the central part of the glandular lobules separately. Microscopy and recording of the results were carried out by two workers independently of each other.

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