Cytospectrofluorometric Characterization of OPT-Induced Fluorescence in Rat Pinealocytes*

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Summary. o-Phthaldialdehyde (OPT) has been employed for the fluorescence histochemical demonstration of histamine. OPT-induced fluorescence with properties different from those of the histamine fluorophore was examined in cells of rat pineal, retina and endocrine pancreas. The fluorescence in the pinealocytes had two excitation maxima, at 370 and 430 nm, respectively. The corresponding emission maxima were at or below 410 nm and at 500 nm. Adjacent mast cells exhibited an identical fluorescence, suggesting that the OPT-reactive compound is released from the pinealocytes. The fluorescence in the retina was of orange colour and confined to the outer nuclear layer. The excitation maxima were at 380 and 410 nm, and the emission maximum was at 575 nm. A blue, OPT-induced fluorescence could be demonstrated in the pancreatic A₂-cells. Cytospectrofluorometric analysis revealed two excitation maxima: 370 nm and 420 nm. The corresponding emission maxima were 430 and 490 nm, respectively. The A₂-cell fluorophore was very resistant to diffusion caused by the hydration in the histochemical reaction. It was also quite UV-stable, in contrast to the histamine fluorophore which is highly diffusible and UV-labile. The OPT-reactive compounds in rat pineal extracts were analyzed by ion-exchange chromatography. It was possible to establish the presence of OPT-fluorescent compounds not identical with histamine. It is evident that the identity of histamine at histochemically detectable sites has to be confirmed by cytospectrofluorometric and chemical analysis.

Introduction

The fluorometric assay for histamine involves its condensation with o-phthalaldialdehyde (OPT) (Shore, Burkhalter and Cohn, 1959). This reaction has been adapted for the fluorescence microscopic demonstration of histamine in both mast cell and non-mast cell stores (for references, see Björklund, Falck and Owman, 1972). The spectral characteristics of the OPT-induced histamine fluorophores have been defined in tissues as well as in various model systems (Ehinger and Thunberg, 1967; Håkanson, Juhlin, Owman and Sporrong, 1970; Tanaka, Giamman, Bensch and Felsenfeld, 1970). However, in the test tube OPT is known to give fluorescence with compounds other than histamine. Such compounds include histidine, peptides with NH₂-terminal histidine (Håkanson, Rönnberg and Sjölund, 1974), spermidine (Kremsner and Pfeiffer, 1966; Håkanson and Rönnberg, 1973), arginine and agmatine (Cohn and Shore, 1967). OPT-induced fluorescence has been described in the cells of the retinal outer nuclear layer (Öhman and Shelley, 1968) and in the pancreatic A₂-cells (Ehinger, Håkanson, Owman and Sporrong, 1968; Takaya, 1970; Brody, Håkanson, Lundquist, Owman and Sundler, 1973) where it could not be linked with histamine. In the case of the A₂-cells it has been

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possible to explain the fluorescence by the presence of a peptide with NH₂-terminal histidine (Håkanson, Owman and Sundler, 1972), namely glucagon (Brody, Håkanson, Lundquist, Owman and Sundler, 1973). In the present report we describe an OPT-induced fluorescence in rat pinealocytes. Its spectral properties are compared with those of the OPT-induced fluorescence at other sites, namely pancreatic islets and retina as well as histamine-storing cells, such as mast cells and endocrine cells in rat stomach (Håkanson, Juhlin, Owman and Sporrong, 1970).

Materials and Methods

Adult albino rats of either sex were killed by decapitation, and the pineal gland, the eyes, and pieces from the pancreas were immediately dissected out. Bovine pineals were obtained from the slaughterhouse within a few minutes after the animals were killed. The oxyntic gland area of the rat stomach was also used for comparison. The tissue specimens were placed on the chuck of a cryostat (Cryo-Cut, American Optical Company) and frozen to the temperature of liquid nitrogen in a propane-propylene mixture. Sections (10–20 μm thick) were cut at −36°C, placed on cover-slips without thawing and freeze-dried over phosphorous pentoxide in a desiccator, kept in the cryostat and evacuated with a mechanical vacuum pump (Edwards ED 50). On the following day, the sections in the desiccator were brought to room temperature and then treated for 90 sec at room temperature with OPT vapour (generated from a few mg of OPT crystals at 100°C for 5 min in a closed glass jar), followed by gentle hydration and subsequent drying (Ehinger and Thunberg, 1967; Håkanson and Owman, 1967). The sections were mounted in xylene or Entellan (Merck) and examined in a fluorescence microscope (light source: Hg lamp; primary filter: Schott UG 1; secondary filter: Schott GG 9) or analyzed unmounted in a modified Leitz microspectrograph (Björklund, Ehinger and Falck, 1967; see below).

Cytospectrofluorometry. Excitation spectra were recorded with a xenon high pressure lamp (XBO 150) without lamp filter, and the 365 nm or 405 nm lines of a high pressure mercury lamp (HBO 200 W/2) were used for recording the emission spectra (lamp filters: Schott UG 1 or BG 12, respectively). In the recordings of the excitation spectra, a barrier filter with cut-off below 510 nm was used. In the emission recordings with the 405 nm lines the barrier filter had a cut-off below 430 nm. The optical system included cardiod dark-field immersion oil condensers of quartz (for recording of the excitation spectra) or glass (for the emission spectra), and an apochromat 25× (NA 0.65) objective. The spectral curves were recorded with an x−y recorder and corrected as previously described (Björklund, Ehinger and Falck, 1968). Results are expressed as relative quanta versus wave-length.

Chemical Analysis. Pineals from the rat (at least ten pineals were pooled) or cow (usually single pineals) were glass-homogenized in 1–2 ml 0.1 N hydrochloric acid; the homogenate was centrifuged at 10000 × g for 10 min. The supernatant was evaporated to dryness in a Rotavapor. The dry residue was taken up in 2 ml re-distilled water and either extracted with a 3:2 mixture of n-butanol and chloroform (Håkanson, 1963) or transferred to a Dowex 50-X4 column (200 mesh, column dimension 13 mm × 20 mm), pretreated with 10 ml of 0.5 M Na₂HPO₄ followed by 5 ml re-distilled water. After passage of the extract, the column was washed with 36 ml 0.1 M Na₂HPO₄, followed by 10 ml 0.1 N NaOH. The flow rate was 0.25 ml/min, 2-ml fractions were collected and analyzed for OPT-reactive compounds by fluorometry. Each fraction was mixed with NaOH to a pH of about 12.5, and 0.1 ml of 0.2% re-crystallized OPT in methanol was added. Final volume was 2 ml. Reaction was run at 25°C for 4 min or at 0°C for 40 min (Håkanson, Rönneberg and Sjölund, 1972). The samples were assayed fluorometrically before and after acidification (to pH 2.5) with 0.5 N H₂SO₄. The same procedure was used with the butanol-chloroform extracts. For details see Results.

Results

A blue OPT-induced fluorescence was demonstrated in the A₂-cells of the pancreatic islets. This fluorophore was markedly resistant to diffusion in the