Inclusion bodies in pinealocytes of the cotton rat (*Sigmodon hispidus*)

An ultrastructural study and X-ray microanalysis

Michal Karasek\(^1\), Nancy K.R. Smith\(^1\), Thomas S. King\(^1\), Larry J. Petterborg\(^1\), John T. Hansen\(^1\), and Russel J. Reiter\(^1\)

\(^1\) Department of Anatomy, University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA;
\(^2\) Laboratory of Electron Microscopy, Department of Pathological Anatomy, Institute of Pathology, School of Medicine, Lodz, Poland

Summary. Pinealocytes of the cotton rat (*Sigmodon hispidus*) often contain large (2–6 μm diameter) intracytoplasmic inclusions, the function of which is not known. These inclusions may represent nucleolus-like bodies, mineral deposits, secretory products or viral inclusions. In this study these inclusions were classified as type A, B or C inclusions based on the amount of electron-dense material interspersed within the finely granular material comprising the bulk of these inclusions. Each type of inclusion was analyzed by X-ray microanalysis and enzymatic proteinaceous digestion. X-ray microanalysis of these inclusions differed both quantitatively and semiquantitatively from that of human or gerbil pineal concretions, the latter two of which are extracellular deposits. Pronase, a proteolytic enzyme, digested the electron-dense material only after longer times of tissue exposure to this enzyme in contrast to the easily digested, finely granular matrix-like material of these inclusions. Such intrapinealocytic inclusions have only been observed in the cotton rat. Their functional significance remains unknown.

Key words: Pinealocytes – Cotton rat – X-ray microanalysis – Protein digestion – Inclusion bodies

In most studies dealing with the ultrastructure of the mammalian pinealocyte, laboratory animals have been used. The pineal gland is believed to

Send offprint requests to: Dr. Thomas S. King, Department of Anatomy, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas 78284, USA

Acknowledgements: The authors wish to thank Ms. Gwynne Duke for her excellent technical assistance and Ms. Nora Dimas for typing the manuscript. This study was supported by NSF grant No. PCM 8003441 to R.J.R. and by a grant from the Polish Academy of Sciences, within project 10.4 to M.K. J.T.H. is the recipient of NIH RCDA KO4 HL-00680
be involved principally in long-term adaptation of reproductive function to environmental lighting conditions (see Reiter 1978). Accordingly, a number of reports on the ultrastructure of pinealocytes of seasonally breeding animals living under natural photoperiods have appeared recently in the literature. In pinealocytes of one such animal, the cotton rat (Sigmodon hispidus), highly unusual cytoplasmic inclusions are consistently present (Matsushima et al. 1979; Karasek et al., in press). Although many pinealocytes in this species possess such inclusions, their functional significance is unknown. Therefore, in this study we examined these inclusion bodies in pinealocytes of the cotton rat using an ultrastructural, X-ray microanalytical and enzymatic digestion approach in an attempt to characterize more specifically these enigmatic organelles.

Materials and methods

Eight cotton rats (six males and two females) were captured by live traps in San Antonio, Texas, during October–November, 1981, and sacrificed on the same day by decapitation, between 13.00 and 14.00 h. All pineal glands were immediately removed and immersion-fixed in 3.5% glutaraldehyde + 2% formaldehyde in 0.067 M cacodylate buffer (pH 7.2; 4° C) for 2 h. Six pineals were postfixed in 1% osmium tetroxide, dehydrated in a graded series of acetone solutions and embedded in Spurr’s (1969) low-viscosity epoxy resin. The remaining two glands were embedded in Epon without postfixation. For the ultrastructural study, thin sections were stained with uranyl acetate and lead citrate and examined with a Siemens 1A electron microscope.

In addition, enzymatic digestion was performed on sections from these embedded glands using the following procedure (Monneron 1966; Monneron and Bernhard 1966). Ultrathin sections were collected on nickel grids, oxidized for 30 min in a 10% periodic acid solution, rinsed in distilled water and incubated in a 1% pronase solution (Protease type XIV, 6 units per mg solid; Sigma Chemical Co., St. Louis, MO) in distilled water (pH 7.4, adjusted with 0.01 N NaOH) for 1, 2, 3, 4, 5, 8, 12 and 24 h. Control sections were treated identically except that the last solution did not contain pronase. Sections were then washed with distilled water and stained with uranyl acetate and lead citrate.

For X-ray microanalysis sections (approximately 250 nm in thickness), obtained from tissue with or without postfixation in osmium tetroxide, were collected on 180 mesh carbon-coated nylon grids and examined unstained with a JSM-35 scanning electron microscope. The grids were inserted into the microscope in a specimen holder constructed of beryllium. The tissue was imaged in the STEM mode at 25 kV accelerating voltage, at a working distance of 39 mm. The probe current was standardized by using the condenser lens setting to set the specimen current to 2.0 x 10^-10 amp with the probe on the beryllium holder. A thick disc tantalum aperture was used beneath the usual molybdenum (Mo) thin foil objective aperture in order to absorb Mo X-rays generated from the Mo foil and thus minimize wide-area extraneous excitation of the sample by X-ray fluorescence. The Si(Li) X-ray detector (Tracer X-ray, Mt. View, California) was positioned 1.5 cm from the specimen with a takeoff angle of 40°. Spectra were collected from 100 to 400 sec using a rapid area scan or a spot mode at a magnification (20000 × -90000 x) sufficiently high to encompass the feature of interest. Spectra were processed and stored by an NS-880 (Tracer Northern, Middleton, Wisconsin) X-ray analysis system with a resolution of 20 eV per channel. The Tracer Northern Super ML multiple least squares fitting routine was used to deconvolute the spectra and to compute integrated peak counts for the individual elements. Spectra were plotted using a 7034A X-Y plotter (Hewlett Packard, Palo Alto, California). At least eight spectra for each type of inclusion body were analyzed.