Scientific Works. Series C. Veterinary Medicine. Vol. LXXI (2), 2025
ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295

RESEARCH REGARDING THE FUNCTION AND STRUCTURE OF THE PINEAL GLAND OF THE PIG

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Abstract

Research on the pineal gland in pigs has gained significant interest due to the physiological similarities between pigs and humans, making pigs a relevant animal model for studying pineal function and its potential applications in medicine.
All though recent studies have focused on the pineal gland (mostly regarding structure and innervation of the gland), our own research highlighted in the present paper, as a result of optical and electronic microscopy (TEM and SEM), details of histological and ultramicroscopic organization of the constituent cell types of the pineal gland in pigs. We focused on demonstrating the possible physiological mechanisms of secretion, as well as the elimination pathways of the active endocrine biological products (indole and peptide hormones) of the component cells of the pineal gland.
Our research significantly contributes with important information about the function and the internal cell mechanisms of this important endocrine gland and provides valuable insights for biomedical applications that can translate in human medicine.

Key words*: electro-microscopy, light pathway, pineal gland, pinealocytes, secretion.*

INTRODUCTION

The use of the pig as an animal model continues to shed light on the complex role of the pineal gland in health and disease. We have highlighted and demonstrated, through the iconography that illustrates this work, a series of aspects regarding the pineal gland, that has yet to be investigated in debt.
The pineal gland in pigs is covered by a very thin conjunctivo-vascular capsule, derived from the pia mater, which presents an abundance of smooth muscle fibers, elastic and reticulin fibers.
It has an elongated shape and is located between the superior colliculi, the habenular commissure and the stria medullaris thalami (Fabris et al., 2004). “This organ is involved in the circadian control and other bodily functions, such as the reproductive cycle, thermoregulation, cardiovascular regulation, pressure and regulating the immune system,, (Silva et al., 2015). Its endocrine secretion is influenced by the amount of light, respectively the night/day cycle, through the light influxes that stimulate the retina and generate excitations through the inferior optic tracts, then joins the median fasciculus of the telencephalon, the light stimulus is finally propagated to the rostral region of the cerebral subcommissural organ in the vicinity of the pineal gland.
The pineal endocrine complex is made up of the pineal gland, the fibroconjunctive fibers originating from the capsule and the pineal recess area corresponding to the subcommissural organ (that Maklenburg and Dimitrova first described in humans), made up of modified secretory ependymal cells. The subcommissural organ (SCO) is one of the circumventricular organs (CVO) - Area Postrema, Median Eminence and Neurohypophysis, Subcommissural Organ, Pineal Gland and Choroid Plexus (Kumar & Singh, 2010; Bon et al., 2024).
“The close spatial relationship between the pineal body and the subcommissural organ (SCO) has been noted by all investigators of the diencephalic roof and in the ontogenetic development, no clear-cut borderline is visible

between the primordia of the pineal gland and the SCO" (Oksche & Korf, 1993).
The in-depth and continuous study of the detailed anatomy of the pineal gland in pigs is of fundamental importance and is built on our previous work (Crînganu et al., 2000; Negreanu et al., 2015).
This research is not merely a descriptive exercise; it serves as a crucial step in the advancement of comparative neuroscience and endocrinology. By meticulously documenting the gland's structure and its connections, we establish a foundational anatomical reference.
This solid reference is essential for guiding future functional investigations. A detailed anatomical description allows for the formulation of more accurate hypotheses about how the cells and neural networks within the pineal gland produce and release hormones, such as melatonin, and how these regulate circadian rhythms, reproduction, and immunity (Jürgen & George, 2012; Shi et al. 2013).
Building on Rodriguez et al. studies in 2005 that concluded that: "tanycytes are bipolar cells bridging the cerebrospinal fluid (CSF) to the portal capillaries and may link the CSF to neuroendocrine events", "using both fluorescent and electron microscopy, Pasquettaz et al. (2021) observed that tanycyte protrusions contain ribosomes, mitochondria, diverse vesicles, and transporters, suggesting dense tanycyte/neuron and tanycyte/blood vessel communications".
Thus, ongoing investigations into the anatomy of the pineal gland in pigs directly contribute to a better understanding of its complex functions, paving the way for significant clinical and biological discoveries.

MATERIALS AND METHODS

Experimental animals: suckling piglets and prepubescent female pigs, crossbred Landrace x Great White.
Each harvested piece was individualized, the data obtained being completed in the study sheet.
The harvesting of the entire diencephalic area was imposed by the existence of intimate connections of the pineal gland with the meningeal layers, mainly the pia mater, which determined the concomitant sampling of adjacent tissues, which were subsequently detached during the processing process.
The pieces were fixed in Baker or Bouin histological solution. Some of the pieces were fixed immediately after excision in glutaraldehyde for processing for microscopy - TEM (Transmission Electron Microscopy) and SEM (Scanning Electron Microscopy).
Sample processing was performed both by usual histological methods (HEA trichrome staining, HE bichrome staining), and by silver impregnation (Gomori staining). The examination was performed and the images were recorded using the device Jeol Multipurpose Analytical S/TEM

RESULTS AND DISCUSSIONS

Following the examination of the histological samples collected by us, containing the pineal gland together with the dorsal portion of the third cerebral ventricle from 10-day-old piglets, we highlighted by optical microscopy, the following structures:

- The capsule of the pineal gland in pigs is fibroconjunctival, being composed of fibroblasts, collagen and reticulin fibers, smooth muscle fibers, which demonstrate its contractile capacity, as well as unmyelinated neurovegetative nerve filaments and blood capillaries that penetrate the parenchyma:

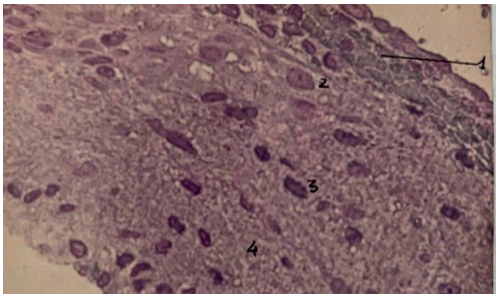

Figure 1. Semi-thin section through the pig pineal gland (Toluidine blue x 40)
1 - gland capsule; 2 - Type I pinealocytes (Light); 3 - Type II pinealocytes (Dark); 4 - gland stroma

- The parenchyma of the pineal gland highlighted by us by optical microscopy, is composed mostly of pinealocytes, secretory nerve cells and mesenchymal interstitial cells with a supporting, nourishing and defensive role, similar to neuroglia.

From a cytological point of view, pinealocytes present a light cytoplasm (Type I pinealocytes) or a dark cytoplasm (Type II pinealocytes), depending on the presence of an increased or decreased/absent amount of hormonal secretion.

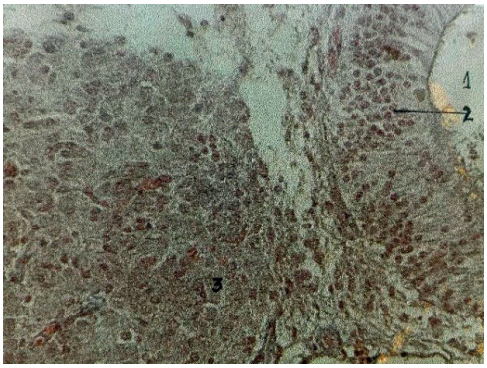

Figure 2. Section through the basal zone of the pineal gland in a pig (HEA x 400)
1 - pineal recess; 2 - ependymal neuroglia; 3 - pinealocytes with accumulations of intercytoplasmic secretion

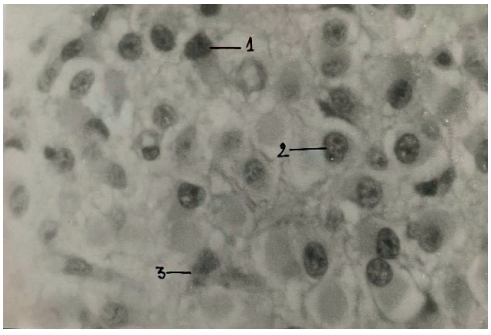

Figure 3. Pinealocyte polymorphism (HEA x 1000)
1 - nucleus and nucleolus of Type II pinealocytes (Dark); 2 - eccentric nucleus of Type I pinealocytes (Light); 3 - neuroglial cells

The stroma of the pineal gland contains interstitial cells similar to astroglia and microglia located intercellularly in the pinealocytes, but also perivascular in the capillaries, along with mast cells with an immune defense role, as well as rare melanocytes and modified nerve cells (Figure 4).

The pineal recess or epiphyseal recess is lined with ependymal cells, arranged in a multilayered manner, corresponding to the specialized ependymal cells which becomes elongated into a layer(s) of columnar epithelium in the subcommissural organ and the tanycytes, (Langlet F., 2013) another type of specialized ependymal cells of the median eminence - also part of the CVO group (Szathmari, A et al. 2014).

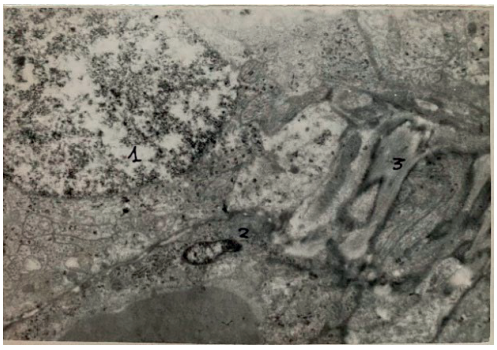

Figure 4. The structure of the stromal cell (fibroblast) in the pineal gland (TEM x 9.100)
1 - Type I pinealocyte (Light); 2 - Fibrocyte in the gland parenchyma; 3 - Extracytoplasmic longitudinal matrix fibers formed from tropocollagen

Results regarding the synthesis and release of the pineal gland secretion in pigs

The TEM examination revealed:

- Type I (Light) pinealocytes represent over 90% of the cells in the pineal gland, containing a large, light euchromatic nuclei, clear cytoplasm and secretory vesicles belonging to the Golgi apparatus, a well-developed smooth endoplasmic reticulum (SER), with a multitude of mitochondria and free ribosomes (Figure 5). “The adrenergic receptors on pinealocytes activate aralkylamine N-acetyltransferase that converts 5-hydroxytryptamine (5-HT, serotonin) to N-acetylserotonin, the precursor of melatonin” (Lee et al., 2021). This explains the presence of serotonin in the secretory vesicles.
- Type II (Dark) pinealocytes with smaller heterochromatic, darker nuclei, dark cytoplasm and a large number of ribosomes, both free and attached to the cisternae of the rough endoplasmic reticulum (RER), and a well-represented Golgi apparatus, with large vacuoles (Figure 6). These are grouped in organized populations both at the level of the glandular parenchyma, arranged around the sinusoidal blood capillaries, and in the vicinity of the pineal recess.

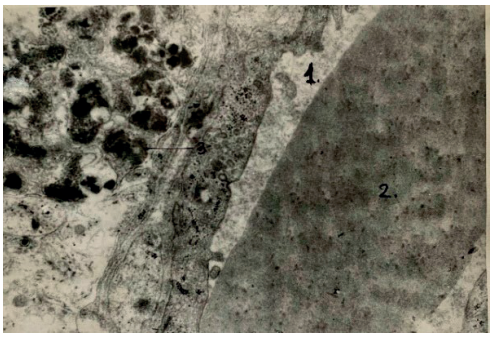

Figure 5. The process of hormonal secretion in type I pinealocytes (light)
1 - capillary lumen; 2 - red blood cell; 3 - dense center secretory vesicles in exocytosis

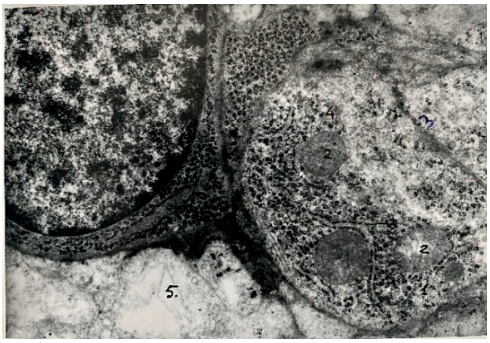

Figure 6. The phenomenon of intra-RER protein secretion in type II pinealocytes in the pig (TEM x 7100)
1 - perimitochondrial polyribosomal zoning; 2 - mitochondria without cristae and with mitochondrial vacuolations; 3 - RER cisterna; 4 - Free polyribosomes in active synthesis process; 5 - Clear electron-transparent secretory vesicles

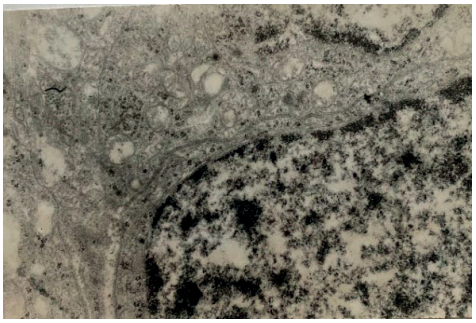

Figure 7. Intercellular boundary of Type II pinealocytes (TEM x 100)

These pinealocytes contain polypeptide hormones (epithalamin) insoluble in lipids and water soluble and melatonin, an indoleamine hormone that is lipid-soluble as well as water-soluble, therefore it appears invisible after the piece is treated for microscopic examination.

The TEM examination confirmed both the GAP junctions between Type I pinealocytes that mediate cell-to-cell exchange (Figure 7), and the contiguity relationships between the pinealocytes and the intraparenchymal capillaries that plead for a distinct endocrine secretion path through cytoplasm extensions of the pineocyte that is close to the capillaries and sinusoidal vessels of the pineal parenchyma (Figure 8)

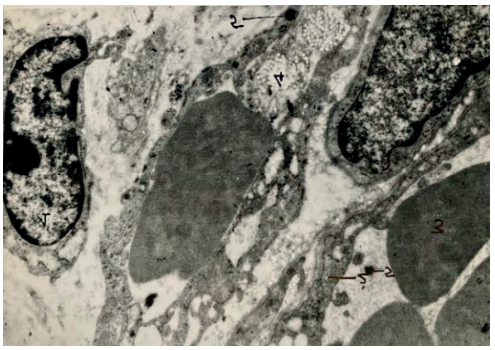

Figure 8. Relationship of type II pinealocyte to blood capillary (TEM x 7100)
1 - Type II pinealocyte; 2 - blood capillary endothelium; 3 - red blood cells; 4 - fibrocyte with tropocollagen fibers; 5 - intracapillary osmiophilic secretory granule

While studies have been conducted regarding the electro microscopic aspects of pinealocytes in general, especially on laboratory animals, data on the aspects of pig pineal cytoarchitecture obtained by SEM, are still in their infancy. The value of the information and understanding of processes at this level, in the pineal gland of the pig, will be determined by the correlation with human medicine, as this subject evolves.

The research presented in this paper completes and confirms, by SEM, the dual pathway of elimination of hormonal secretion. The mechanism of type I pinealocytes is based on the formation of electrode-dense osmiophilic vesicles in the sinusoidal blood capillaries of the glandular parenchyma, and the mechanism of type II pinealocytes is based on paracrine secretion at the level of the epiphyseal recess and from there into the third ventricle through the cerebrospinal fluid.

The pineal gland is very active in the pre-puberty period, during which, the pinealocytes secretion of high levels of polypeptide hormones (epithalamin) leads to a high synthesis of serotonin, that converts to melatonin inhibiting

the anterior pituitary gonadotropins (Wenqi et al., 2019).
Also, the day-night cycle functionally influences the hormonal reactions in the body, following the path of the optical receptors, respectively the retina - optic nerve - optic chiasm - intracerebral optical pathways whose threads reach the structure of the pineal gland (Crînganu et al., 1995).

Figure 9. Exocytosis of osmiophilic vesicles from type I pinealocytes (SEM x 6200)
1 - buttoned terminal cytoplasmic extension of type I pinealocyte at the endothelium of a blood capillary; 2 - blepsoid relief elements marking the moment of exocytosis of endocrine secretory vesicles at the capillary level; 3 - endothelial cells of sinusoid blood capillary in pineal parenchyma

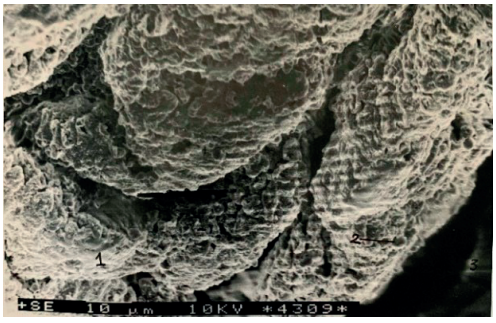

Figure 10. SEM image of the pineal recess in the pig (SEM x 2100)
1 - ependymocytic membrane reliefs; 2 - hormone secretion vesicles in transdiapedesis; 3 - the pineal recess communicating with the CSF-filled third ventricle

Photoexcitation induced by intense light inhibits pineal secretion (melatonin), inducing the release at the hypothalamus level of Gonadotropin-Releasing Hormones (Gn-RH) that determine the secretion of the pituitary gonadotropins (Crînganu et al., 1995).
With puberty, due to the involution of the pineal gland, it no longer has an inhibitory role on the hypothalamus, which increases Gn-RH level leading to the development the sexual hormones. However, the pineal gland's antigonadotropic role is partially preserved, when in the spring, a season with strong light inhibits the remaining secretion of the pineal gland and allows the entry in to the reproductive period for animals.
The secretion mechanisms of the pinealocyte hormones are endocrine at the level of capillaries (Figure 9) for hormones with a metabolic role, but the indoleamine hormones secretion is in the IIId ventricle, in the cerebrospinal fluid (CSF) through the pineal recess (Figure 10).

CONCLUSIONS

The pineal gland in pigs benefits from a rich vascularization formed by numerous fenestrated sinusoidal capillaries in which Type II (Dark) pinealocytes discharge their secretion through specific cytoplasmic extensions (similar to synaptic terminal buttons)
Transmission electron microscopy (TEM) investigations revealed a different proportion of organelles in the two observed pinealocyte subpopulations, namely a wealth of rough endoplasmic reticulum and Golgi apparatus in Type II (Dark) pinealocytes and clear cytoplasm, secretory vesicles belonging to the Golgi apparatus, numerous mitochondria associated with a developed smooth endoplasmic reticulum in Type I (Light) pinealocytes.
The specific evolution of the organelles of each type of pinealocyte is a consequence of the two distinct and clear mechanisms of secretion: an endocrine pathway through the capillary system and the cerebrospinal fluid pathway.
The pinealocyte complex has the function of mixed indoleamine secretion and polypeptides with a direct regulatory role of hypothalamic-pituitary neurosecretion and indirect effect on the thyroid and adrenal glands, ovaries and testicles

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