Ultrastructural and Immunocytochemical Studies of the Viscacha (*Lagostomus maximus maximus*) Pituitary Pars Tuberalis

EDITH PEREZ ROMERA,[1]* FABIAN MOHAMED,[1] VERÓNICA FILIPPA,[1] TERESA FOGAL,[2] SUSANA DOMINGUEZ,[1] LUIS SCARDAPANE,[1] AND RAMÓN PIEZZI[2]

[1]Cátedra de Histología y Embriología, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, San Luis, Argentina
[2]Instituto de Histología y Embryología (IHEM), Universidad Nacional de Cuyo, Consejo Nacional de Investigaciones (CONICET), Argentina

ABSTRACT

The hypophyseal pars tuberalis (PT) has been the focus of numerous studies attempting to understand its physiological role in the reproductive regulation and modulation by the neuroendocrine system. Ultrastructural studies of the PT in a number of species have shown that it consists of a well-developed hypophyseal area with important secretory activity, demonstrated by the abundance of secretory granules in the cytoplasm and the marked blood irrigation. This article describes ultrastructural and immunocytochemical aspects of the PT in viscachas captured in their habitat. The cell types identified were PT-specific cells, agranulated cells, and Folliculostellate cells. PT-specific cells are divided into type I and II. Type I cells have cytoplasms with secretory granules of 150–500 nm diameter. The secretory granules of type II PT-specific cells are 65–200 nm in diameter. Both cellular types exhibit numerous nerve endings on the plasmatic membranes. Agranulated cells exhibit nuclei with lax chromatin, mitochondria, phagosomes, scarce Golgi complex, and rough endoplasmic reticulum. Folliculostellate cells exhibit an irregularly shaped and moderately condensed nucleus. All the described cellular types exhibit deposits of cytoplasmic glycogen. The immunocytochemical study revealed the presence of cells immunostained for LH-β and FSH-β in the PT caudal zone. ACTH was only detected in the zona tuberalis. No staining was observed with antiprolactin, anti-TSH-β, and anti-GH sera. Folliculostellate cells exhibited staining with anti-S-100. The results demonstrate that the viscacha PT is a hypophyseal zone with specific cellular types, which exhibits evident secretory activity. The presence of nerve endings suggests neural control of the function of PT cells.

Key words: *Lagostomus maximus maximus*; pituitary; pars tuberalis; ultrastructure; immunocytochemistry

In recent years, the hypophyseal pars tuberalis (PT) has been the object of numerous studies attempting to elucidate its histophysiology and hormonal role in the neuroendocrine system, which regulates and modulates important functions such as reproduction (Fitzgerald, 1979; Wittkowski et al., 1992; Morgan, 2000; Guerra and Rodríguez, 2001).

The ultrastructural characteristics of the PT in most of the species studied show that it consists of a well-developed hypophyseal area with important secretory activity, demonstrated by the presence of secretory granules and abundant irrigation. Studies performed in mammals, birds, and amphibians have demonstrated in all cases a well-defined PT histoarchitecture (Oota and Kurosomi, 1966; Dellman et al., 1974; Fitzgerald, 1979).

The PT of mammalian species extends from the median eminence and surrounds the infundibular stalk to the

Grant sponsor: Universidad Nacional de San Luis; Grant number: Proyecto 22/Q303 CyT.

*Correspondence to: Edith Perez Romera, Cátedra de Histología y Embriología, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Av. Ejército de los Andes 950-2° Piso, (5700) San Luis, Argentina. Fax: 54-2652-422644. E-mail: eperezro@unsl.edu.ar

Received 7 June 2004; Accepted 19 October 2004
DOI 10.1002/ar.a.20163
Published online 25 March 2004 in Wiley InterScience (www.interscience.wiley.com).

anterolateral zone of the pars distalis (PD) (Gross, 1984). The secretory cells are in close contact with the capillaries of the primary plexus of the portal hypothalamic-hypophyseal system, together with nerve endings of fibers coming from the neuroendocrine neurons of the hypothalamus, suggesting a probable modulation of PT secretory cells on the PD glandular cells (Wittkowski et al., 1999). The PT differs from the PD both structurally and morphologically. The PT is a bi- or multilayered region formed by specific secretory cells (the so-called PT-specific cells), folliculostellate cells, and migratory cells coming from the PD (Oota and Kurosomi, 1966; Cameron and Foster, 1972; Stoeckel and Porte, 1984). The diameter of the secretory granules differs according to the species (Dellman et al., 1974)

The presence in the PT caudal zone of hormones produced in the PD, particularly LH, FSH, and TSH, has been demonstrated in some species by immunocytochemical techniques (Baker and Yu, 1975; Girod et al., 1980; Asa et al., 1983; Gross, 1984; Böckers et al., 1996). These endocrine products are considered to be synthesized by migratory cells coming from the PD. Certain reports indicated that PT-specific cells do not interact with any PD-specific antisera (Baker and Yu, 1975; Gross, 1984; Böckers et al., 1994). However, the expression of the α-subunit of glycoprotein hormone chains has been demonstrated. The fact that only the common α-subunit is expressed in the PT cells is explainable by the fact that PT cells start to express PTX-1 during pituitary development. However, expression of further necessary genes does not follow (Lanctot et al., 1999). These cells do not react with a specific antibody against the β-subunit of LH (Böckers et al., 1996; Stoeckel et al., 1994). The folliculostellate cells of the PT differ from those described in the PD (Kameda, 1996a, 1996b). All these studies have demonstrated that the PT possesses secretory activity and that its hormones probably modulate the endocrine behavior of some cellular types in the PD parenchyma (Wittkowski et al., 1992; Hazlerigg et al., 1996; Morgan et al., 1996; Lafarque et al., 1998).

The viscacha (*Lagostomus maximus maximus*) is a subterranean rodent belonging to the family Chinchillidae, living in the central zone of Argentina. It inhabits in extensive burrow systems forming large colonies. These herbivorous animals, nocturnally active, emerge from their burrows during periods of darkness from dawn to dusk to feed on the surrounding vegetation (Llanos and Crespo, 1952). In our laboratory, studies have been conducted to determine the structural and ultrastructural characteristics of hypophyseal PD and pars intermedia (PI) (Scardapane et al., 1983; Mohamed et al., 1995). These two hypophyseal regions are characterized by the presence of follicular structures with colloidal material in the lumen and extracellular accumulation of colloid, which probably stores hormonal products (Mohamed et al., 2000). The viscacha has a seasonal reproductive cycle (Fuentes et al., 1991), with important participation of the pineal gland (Dominguez et al., 1987; Cernuda-Cernuda et al., 2003). These changes at the central level affect testicular histophysiology (Muñoz et al., 1997). Thus, this wild rodent might be an interesting model to analyze the PT, on account of its morphofunctional relationship with PD and the reproductive system.

The purpose of the present work is to study the morphological, ultrastructural, and immunocytochemical characteristics of the PT of *Lagostomus maximus maximus* in an attempt to contribute to the understanding of its possible function.

MATERIALS AND METHODS

Animals and Tissue Preparation

Ten adult male viscachas (*Lagostomus maximus maximus*) weighing 4–8 kg were captured in their habitat in February, March, and April of 2001, near San Luis, Argentina (33° 20′ south latitude and 760 m altitude). The animals were anesthetized with Nembutal (pentobarbital) and quickly decapitated. The skull was opened and the basal hypothalamus and hypophysis were rapidly dissected out en bloc. The specimens to be used for light microscopy were fixed in Bouin's fluid for 24 hr, tissue was dehydrated in a graded series of ethanol, cleared in xylene, and embedded in paraffin. Sagittal sections were cut at 5 μm using a Reichert-Jung microtome and stained with hematoxylin-eosin and hematoxylin-PAS.

The glands to be used for electron microscopy were fixed by immersion in Karnovsky's fluid (Karnovsky, 1965), postfixed in 1% osmium tetroxide 2 hr at 4°C, washed in phosphate buffer (pH 7.2–7.4), dehydrated in acetone, and embedded in Spurr plastic resin. Sections of 1 μm were cut with a Porter-Blum ultramicrotome, stained with 1% Toluidine blue, and examined by light microscopy. Ultrathin sections were contrasted with lead citrate and uranyl acetate (Milloning, 1961) and examined under a Siemens Elmiscop I electron microscope.

Immunocytochemical Techniques

The hypophyses were fixed by immersion in Bouin's fluid for 24 hr, dehydrated, and embedded in paraffin. Sagittal sections were cut at 5 μm and serial tissue sections were mounted on gelatin-coated microscope slides (two or three sections per slide).

Immunocytochemical staining was performed using a streptavidin-biotin-peroxidase complex method. Sections were xylol-deparaffinized, hydrated in a decreasing alcohol series, washed in phosphate-buffered saline (PBS; 0.01M, pH 7.6), and treated with 3% H_2O_2 to block endogenous peroxidases. The sections were then washed with PBS and incubated overnight in a moist chamber at 4°C with the primary mouse antisera: antihuman LH-β, diluted 1:100; antihuman FSH-β, diluted 1:200; antihuman TSH-β, diluted 1:100; antihuman PRL, diluted 1:60; antihuman GH, diluted 1:50; antisynthetic human ACTH 1-24, diluted 1:100; and antihuman S-100 protein, diluted 1:200. The antisera used in this study were purchased from BioGenex (San Ramon, CA). The immunocytochemical visualization was carried out using the BioGenex Super Sensitive Ready-to-Use Immunostaining Kit at 20°C; the slides were incubated with the diluted biotinylated antimouse IgG for 30 min, washed in PBS, and incubated for 30 min with horseradish peroxidase-conjugated streptavidin. The reaction site was revealed by 100 μl 3,3′-diaminobenzidine tetrahydrochloride (DAB; BioGenex) in 2.5 ml PBS and 50 μl H_2O_2. The slides were counterstained with hematoxylin for morphological orientation, dehydrated, and mounted.

In order to confirm the specificity of the immunoreactive procedures, adjacent sections were stained according to the protocol described above but incubation in the primary antisera was omitted. No positive structures or cells were found in these sections.

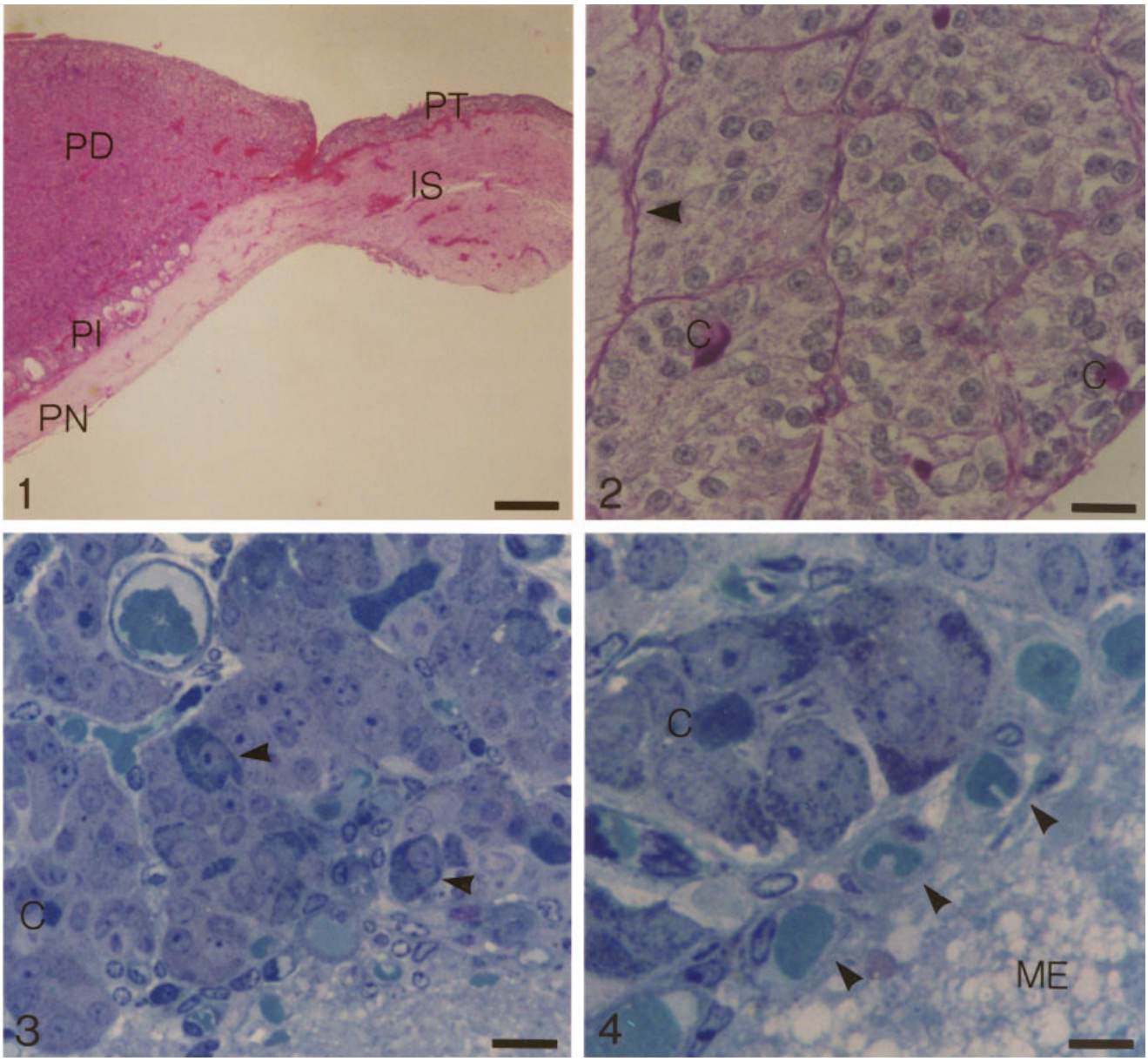


Fig. 1. Morphological characteristics of the viscacha pituitary. PT, pars tuberalis; IS, infundibular stalk; PD, pars distalis; PI, pars intermedia; PN, pars nervosa. Hematoxylin-eosin stain. Magnification = 20×. Scale bar = 0.5 mm.

Fig. 2. Light micrograph of the viscacha pars tuberalis showing the columnar arrangement of cells. The follicular cavity (C) is filled with PAS-positive colloid-like material. Notice the developed capillary plexus (arrowhead). Hematoxylin-PAS stain. Magnification = 400×. Scale bar = 25 μm.

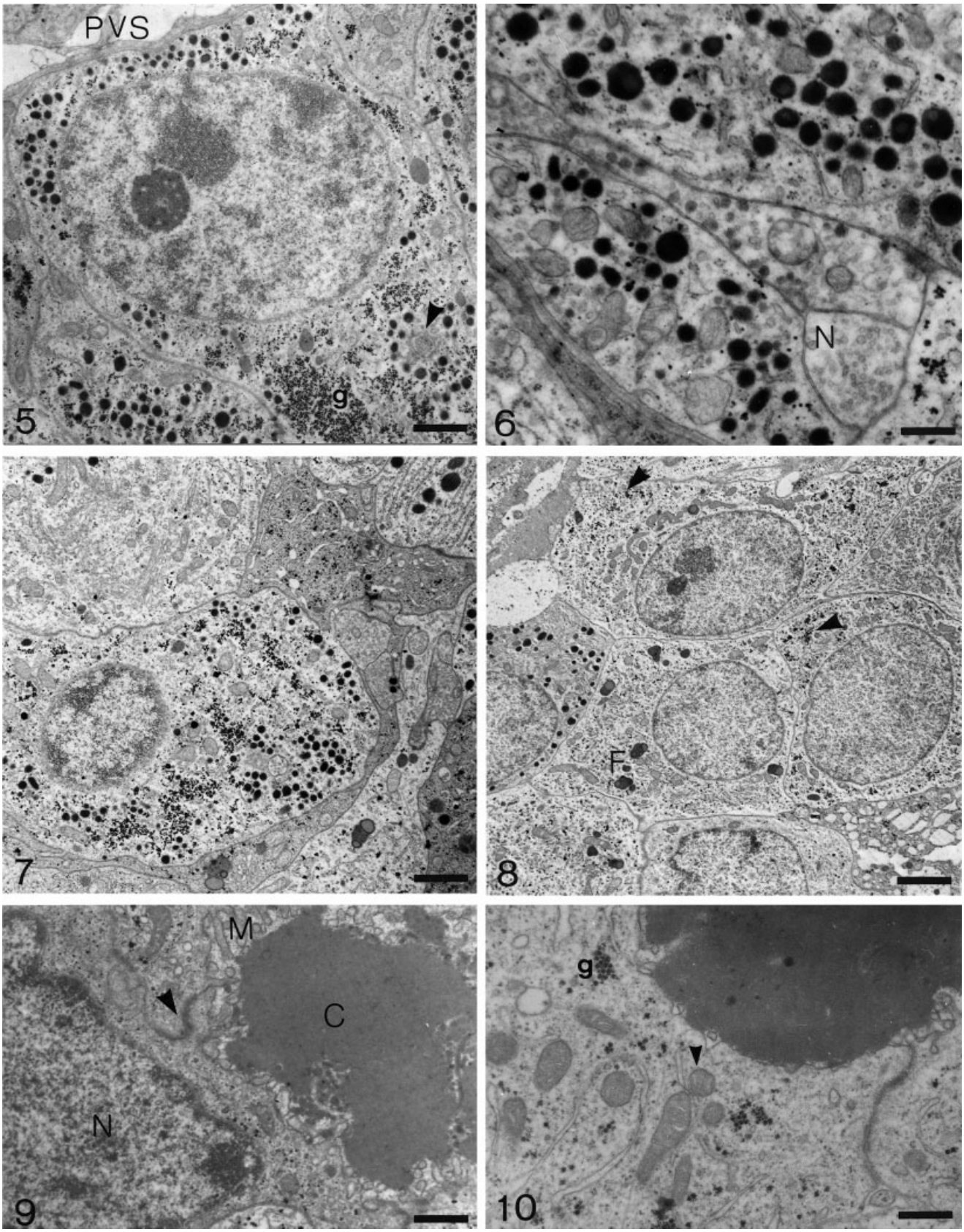
Fig. 3. Light microscopic aspects of the proximal (cephalic zone) pars tuberalis. Semithin section. C, follicular cavity; arrowheads, PT-specific cells. Toluidine blue stain. Magnification = 400×. Scale bar = 25 μm.

Fig. 4. Light micrograph of the viscacha PT showing its follicular structure with colloid-like material inside the lumen. The specific granulated cells exhibit a marked polarization of the granules towards the blood capillaries (arrowheads). C, follicular cavity filled with colloid-like material; ME, median eminence. Semithin section. Toluidine blue stain. Magnification = 1,000×. Scale bar = 10 μm.

RESULTS

Light Microscopy

The hypophyseal PT of the viscacha is a glandular sleeve of numerous cell layers that surrounds the infundibular stalk in its anterolateral region and projects toward the dorsal region where the number of layers decreases significantly. It extends from the hypothalamic median eminence to the rostral zone of the PD. The PT exhibits a well-developed capillary plexus, probably belonging to the hypothalamus-hypophyseal portal system. The capillaries extend all along the hypophyseal zone. Capillarization is even more intense than in the PD. Light microscopy observation of stained sections using routine



Figures 5–10.

histochemical techniques reveals the presence of chromophobe and basophilic cells similar to those described in the PI of the same rodent (Scardapane, 1990).

The PT cells are arranged longitudinally in cords and separated by blood capillaries. The study of the histoarchitecture demonstrated that some cells are organized around a central lumen forming structures with follicular characteristics. The predominant cell type in these formations corresponds to folliculostellate cell. The colloidal material present in the lumen is PAS-positive, which indicates that at least part of its content is formed of carbohydrates, probably as part of larger molecules such as glycoproteins (Figs. 1–4).

Electron Microscopy

Observation with the transmission electronic microscope reveals the presence of specific granulated cells (PT-specific cells), agranulated cells, and folliculostellate cells. Two types of PT-specific cells can be distinguished: cells with large secretory granules (type I), and cells with small secretory granules (type II). Both cell types are in close contact with the membrane of blood capillaries, with secretory granules facing this membrane.

Type I PT-specific cells. These cells exhibit eccentric nuclei, regular edges, and chromatin scattered in the form of small granules. The cytoplasm contains secretory granules of variable size, with diameters between 150 and 500 nm and heterogeneous electrodensity. Additionally, they exhibit large round, oval, and elongated mitochondria, well-developed rough endoplasmic reticulum, Golgi complex, and abundant scattered glycogen. Numerous nerve endings in contact with the plasma membrane are observed in this cell type (Figs. 5 and 6).

Type II PT-specific cells. These cells exhibit an eccentric nucleus with regular edges and finely dispersed chromatin. The electrodense secretory granules have diameters between 65 and 200 nm. They show scarce rough endoplasmic reticulum, round and elongated mitochondria, and abundant glycogen scattered throughout the cytoplasm. Nerve endings in contact with the plasma membrane are also observed (Fig. 7). Both types of granulated cells predominate in the PT cephalic zone, close to the median eminence.

Agranulated cells. These cells have nuclei with finely distributed chromatin. A voluminous cytoplasm exhibits numerous oval and elongated mitochondria, Golgi complex, and scarce rough endoplasmic reticulum, as well as abundant phagosomes and glycogen deposits (Fig. 8). Agranulated cells are distributed along the entire PT.

Folliculostellate cells. These cells present irregularly shaped nuclei, moderately condensed chromatin, and elongated mitochondria. They are arranged in follicles. The plasma membrane exhibits microvilli that project into the lumen, which displays colloidal-like material. Numerous junctional complexes are observed, some of which are of the extended desmosome type. Glycogen was also observed in the cytoplasm, although in lower amounts when compared to the other cell types. The presence of glycogen constitutes the main difference compared with folliculostellate cells of viscacha PD and PI (Figs. 9 and 10)

Immunocytochemistry

The immunocytochemical study revealed isolated or grouped LH-β and FSH-β immunopositive cells only in the caudal part of the PT, in the proximity of the PD. Cells reactive to the anti-ACTH serum were detected only in the zona tuberalis, but not in the PT proper. After incubation with anti-PRL, anti-TSH, and anti-GH, no cells in the PT were stained. S-100 protein was detected in the tuberal parenchyma cells, which present cytoplasmic projections (Figs. 11–14).

DISCUSSION

The morphology of the PT has been studied in a number of species: rat (Oota and Kurosomi, 1966), rabbit (Cameron and Foster, 1972), amphibians (Doerr Schott, 1971; Dellman et al., 1974), chicken (Grignon and Guedenet, 1968; Dellman et al., 1974), monkey (*macaca fascicularis*) (Bock et al., 2001), and guinea pig (Kameda, 1990). While some morphological characteristics are shared between species, others show species-specific variations. Thus, while some cells of the PT exhibit marked features of secretory activity in all the species, their secretory granules vary in size and number (Dellman et al., 1974). In the adult rat, they are scarce; their diameter is between 100 and 120 nm, and the cytoplasm contains a low amount of glycogen. In the mouse, the number of granules is higher than in the rat and the diameter is 140–300 nm, with a greater amount of cytoplasmic glycogen. In the garden door mouse, the diameter ranges from 120 to 180 nm and exhibits glycogen. In the cat, the diameter is between 160 and 230 nm. In cattle, the granules are scarce and their diameter is in the 120–180 nm range. In chicken, the granules are 180–250 nm in diameter, and in newt, 185–230 nm (Dellman et al., 1974). In the rabbit, granule diameter is 90–116 nm, with no presence of glycogen, which constitutes a specific characteristic of this rodent (Cameron and Foster, 1972). In the monkey (*macaca fascicularis*), the diameter is in the 100–120 nm range (Bock et al., 2001). The results obtained in this study show that

Fig. 5. Electron micrograph of type I PT-specific cells with particularly abundant secretory granules. This cell faces the perivascular space (PVS) of a portal capillary. Note the polarization of the secretory granules toward the blood capillaries. g, glycogen particles; arrowhead, nerve ending. Magnification = 4,000×. Scale bar = 2.5 μm.

Fig. 6. Higher magnification of a portion of a type I PT-specific cell. N, nerve ending in contact with the plasma membrane of various glandular cells. Magnification = 10,000×. Scale bar = 1 μm.

Fig. 7. Characteristic ultrastructural image of a type II PT-specific cell with well-developed rough endoplasmic reticulum, small secretory granules intermingled with glycogen particles. A portion of a type I PT-specific cell can be seen in the upper right-hand corner. Magnification = 5,000×. Scale bar = 2 μm.

Fig. 8. Characteristic aspects of agranular cells of the PT of *Lagostomus maximus maximus* with round and elongated mitochondria. Lysosome-like bodies (F) are frequently observed. Arrowheads, glycogen particles. Magnification = 2,000×. Scale bar = 5 μm.

Fig. 9. The follicular cavity (C) is surrounded by folliculostellate cells. Cells exhibit microvilli (M) that project into the lumen. Arrowhead, junction complex; N, nucleus of folliculostellate cell. Magnification = 8,000×. Scale bar = 1.25 μm.

Fig. 10. Another characteristic view of a folliculostellate cell surrounding a follicular cavity filled with amorphous electron dense material. g, glycogen particles; arrowhead, mitochondria. Magnification = 10,000×. Scale bar = 1 μm.

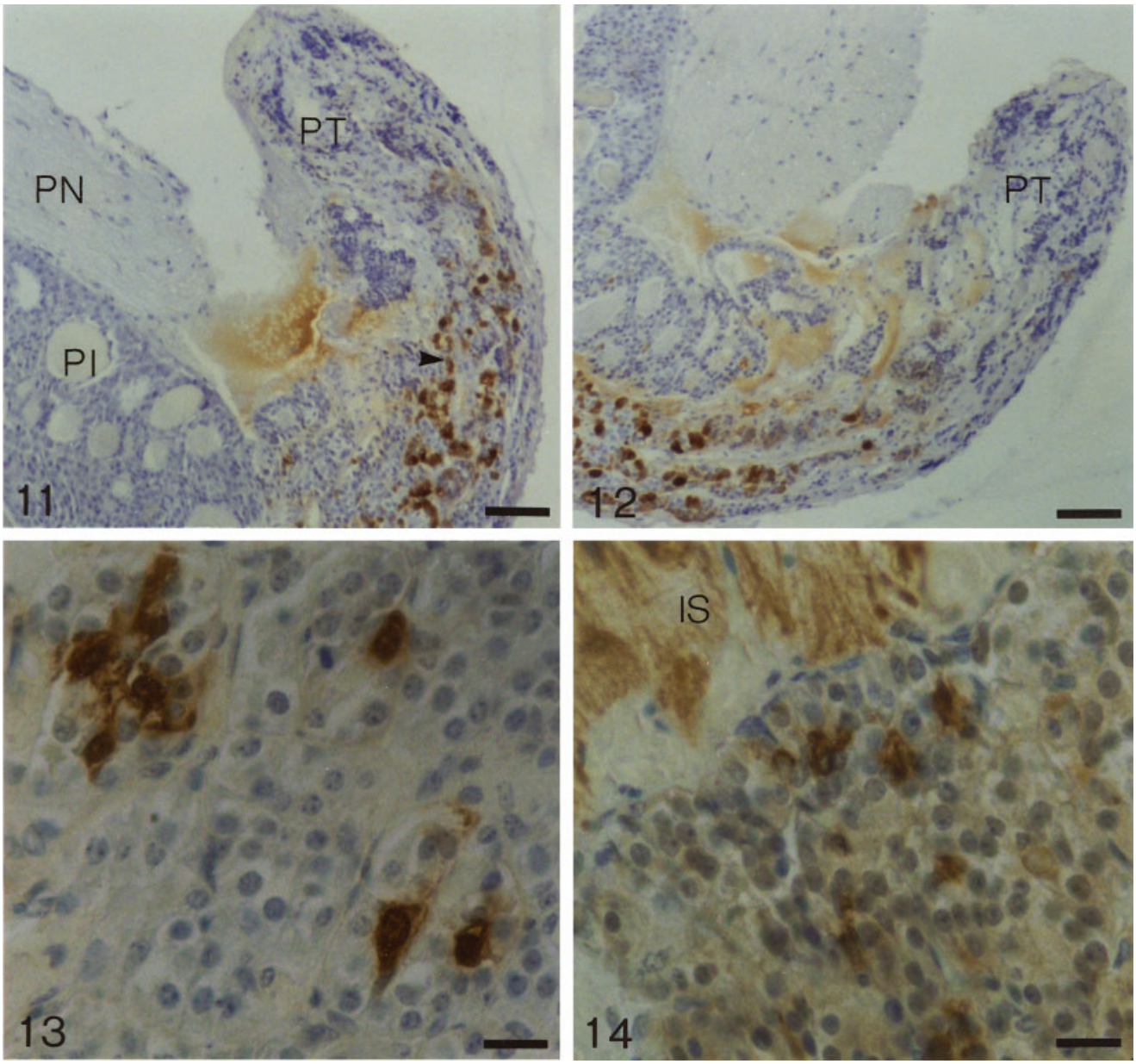


Fig. 11. Midsagittal section of the viscacha hypophysis immunostained with antihuman FSH-β. Immunoreactive cells (arrowhead) are localized in the caudal part of the PT. PT, pars tuberalis; PN, pars nervosa; PI, pars intermedia. Magnification = 100×. Scale bar = 0.1 mm.

Fig. 12. Midsagittal section of the viscacha hypophysis immunostained with antihuman LH-β. The immunoreactive cells could only be detected in the caudal part of the PT in the proximity of the PD. PT, pars tuberalis. Magnification = 100×. Scale bar = 0.1 mm.

Fig. 13. ACTH immunoreactive cells, isolated or grouped in clusters. These cells were detected only in the zona tuberalis. Magnification = 400×. Scale bar = 25 μm.

Fig. 14. Detail of S-100 protein immunoreactive cells within the PT. IS, infundibular stalk. Magnification = 400×. Scale bar = 25 μm.

two types of granulated cell populations can be distinguished in the viscacha PT, one type (type I) characterized by large granules and the other (type II) by small granules. Abundant and scattered deposits of cytoplasmic glycogen are observed in both cell types. It is interesting to note that the granular diameter of type I cells is larger than those determined in PT-specific cells of the other mammalian species.

The cytoplasmic polarization of the secretory granules toward the blood capillaries suggests a rapid release of their content into the primary capillary plexus of the portal hypothalamus-hypophyseal system. This supports the hypothesis that the products synthesized in the PT regulate the secretory activity of some PD cells (Wittkowski et al., 1992; Hazlerigg et al., 1996; Morgan, 1996; Lafarque et al., 1998).

The numerous nerve terminals in close contact with the plasma membrane of the granulated cells suggest hypothalamic control of the release into circulation of these cells' hormonal products. These results give structural

support to previous studies in rat (Johnston et al., 2003). Thus, it is probable that hypothalamic nuclei modulate the synthesis and release of some hormones produced in the PD, using the PT hormones as mediators.

Morgan et al. (1991) have shown that the agranular secretory cells of the ovine PT are of the melatonin responsive-type. Numerous agranular cells were also observed in the PT of the viscacha, a feature that distinguishes it from the PT of other species.

Folliculostellate cells were also observed in the PT parenchyma, forming follicles of varied diameter surrounding the follicular cavity, which has colloidal-like material. The cytoplasmic proximity between these structures and granulated cells suggests that they probably constitute a deposit site of detritus and of products synthesized in the PT-specific cells. This hypothesis is supported by the PAS-positive content of the colloid, which is probably constituted of glycoproteins.

It is important to note that the follicles observed in the PT have characteristics similar to those described in the PD and PI of the same rodent in previous studies, in which we demonstrated the presence of glycoproteins (Scardapane et al., 1983) and the dependence of the number of follicles on the functional state of the gland (Mohamed et al., 2000). Kameda (1990) described the presence of follicles in the guinea pig PT and their modifications with age, suggesting a physiological role for these structures. However, the folliculostellate cells of the PT differ morphologically from those described in the PD and PI, suggesting a specific function in this glandular zone (Kameda, 1996a, 1996b).

The immunocytochemical study of the viscacha PT demonstrated the scarce presence of gonadotrope cells (LH and FSH cells) located in the caudal zone near the PD. This is similar to descriptions in sheep and rat (Gross, 1984; Rudolff et al., 1993; Böckers et al., 1994; Wittkowski et al., 1999). On the other hand, in monkey (*macaca fascicularis*), the gonadotrope cells are distributed along all the PT (Bock et al., 2001). In viscacha, some ACTH cells were detected in the zona tuberalis but not in the PT itself. No immunostaining was detected for the hormones TSH, GH, and PRL. These results suggest that the viscacha PT plays a specific role different from that of the rest of the gland.

The folliculostellate cells observed in the viscacha PT were immunoreactive to the protein S-100, which reveals a typical morphology with cytoplasmic projections that connect large PT zones. These cells may form a network within the hypophysis and may be responsible for paracrine regulation (Fauquier et al., 2001).

The results of this study suggest that the hypophyseal pars tuberalis of the viscacha exhibits a marked secretory activity with a specific function differing from that of the pars distalis. Further studies will be needed in order to elucidate its physiological role and likely participation in the neuroendocrine control system of seasonal reproduction.

ACKNOWLEDGMENTS

The authors thank Mr. J. Arroyuelo and Mr. N. Perez for their technical participation.

LITERATURE CITED

Asa SL, Kovacs K, Bilbao JM. 1983. The pars tuberalis of the human pituitary: a histologic, immunohistochemical, ultrastructural and immunoelectron microscopic analysis. Virch Arch A Pathol Anat Histopathol 399:49–59.

Baker BL, Yu YY. 1975. Immunocytochemical analysis of cells in the pars tuberalis of the rat hypophysis with antisera to hormones of the pars distalis. Cell Tissue Res 156:443–449.

Bock N, Böckers TM, Bockman J, Nowak P, Buse E, Wittkowski W. 2001. The pars tuberalis of the monkey (*Macaca fascicularis*) hypophysis: cell types and hormone expression. Cells Tissues Organs 169:55–63.

Böckers TM, Bockman J, Fauteck JD, Kreutz MR, Bock R, Wittkowski W. 1994. Pars tuberalis-specific cells in the ovine pituitary do express the common α-chain of glycoprotein hormones: an in situ hybridization and immunocytochemical study. Eur J Endocrinol 131:540–546.

Böckers TM, Bockman J, Fauteck JD, Wittkowski W, Sabel BA, Kreutz MR. 1996. Evidence for gene transcriptions of adenohypophyseal hormones in the ovine pars tuberalis. Neuroendocrinology 63:16–27.

Cameron E, Foster CL. 1972. Some light an electron microscopical observations on the pars tuberalis of the pituitary gland of the rabbit. J Endocrinol 54:505–511.

Cernuda-Cernuda R, Piezzi RS, Dominguez S, Alvarez-Uría M. 2003. Cell populations in the pineal gland of the viscacha (*Lagostomus maximus maximus*): seasonal variations. Histol Histopathol 18: 827–836.

Dellman HD, Stoeckel ME, Hindelang-Gertner C, Porte A, Stutinsky F. 1974. A comparative ultrastructural study of the pars tuberalis of various mammals, the chiken and the newt. Cell Tissue Res 148: 313–329.

Doerr Schott J. 1971. La pars tuberalis de *Rana temporaria* L.: cytologie et ultrastructure. Gen Comp Endocrinol 11:516–523.

Dominguez S, Piezzi RS, Scardapane L, Guzmán JA. 1987. A light and electron microscopic study of the pineal gland of the vizcacha (*Lagostomus maximus maximus*). J Pineal Res 4:211–219.

Fauquier T, Guérineau NC, McKinney RN, Bauer K, Mollard P. 2001. Folliculostellate cell network: a route for long-distance communication in the anterior pituitary. Proc Natl Acad Sci USA 98:8891–8896.

Fitzgerald KT. 1979. The structure and function of the pars tuberalis of the vertebrate adenohypophysis. Gen Comp Endocrinol 37:383–399.

Fuentes LB, Caravaca N, Pelzer LE, Scardapane L, Piezzi RS, Guzmán JA. 1991. Seasonal variations in the testis and epididimys of viscacha (*Lagostomus maximus maximus*). Biol Reprod 45:493–497.

Girod C, Dubois MP, Trouillas J. 1980. Immunohistochemical study of the pars tuberalis of the adenohypophysis in the monkey, *Macaca irus*. Cell Tissue Res 210:191–203.

Grignon G, Guedenet JC. 1968. Observation sur l'ultrastructure de la neurohypophyse et de la pars tuberalis chez le poulet au cours de la vie embryonnaire et après l'éclosion. Arch Anat Histol Embryol Norm Exp 51:277–286.

Gross DS. 1984. The mammalian hypophyseal pars tuberalis: a comparative immunocytochemical study. Gen Comp Endocrinol 56: 283–298.

Guerra M, Rodríguez EM. 2001. Identification, cellular and subcellular distribution of 21 and 72 kDa protein (tuberalin?) secreted by specific cells of the pars tuberalis. Endocrinology 168:363–379.

Hazlerigg DG, Hastings MH, Morgan PJ. 1996. Production of a prolactin releasing factor by the ovine pars tuberalis. J Neuroendocrinol 8:489–492.

Johnston JD, Messager S, Ebling FJP, Williams LM, Barrett P, Hazlerigg DG. 2003. Gonadotrophin-releasing hormone drives melatonin receptor down-regulation in the developing pituitary gland. Proc Natl Acad Sci 100:2831–2835.

Kameda Y. 1990. Occurrence of colloid-containing follicles and ciliated cysts in the hypophysial pars tuberalis from guinea pigs of various ages. Am J Anat 188:185–198.

Kameda Y. 1996a. Differential distribution of S-100 protein and vimentin in the hypophyseal pars tuberalis of the guinea pig. J Histochem Cytochem 44:501–510.

Kameda Y. 1996b. Ultrastructural immunogold localization of vimentin and S-100 protein in guinea pig. J Histochem Cytochem 44:511–518.

Karnovsky MJ. 1965. A formaldehyde-glutaraldehyde fixate of high osmolarity for use in electron microscopy. J Cell Biol 27:49A.

Lafarque M, Oliveros L, Aguado L. 1998. Efecto de secreciones de pars tuberalis de la adenohipofisis sobre la liberación de prolactina desde pars distalis. Medicina 58:36–40.

Lanctot C, Gauthier Y, Drouin J. 1999. Pituitary homeobox 1 (Ptx1) is differentially expressed during pituitary development. Endocrinology 140:1416–1422.

Llanos AC, Crespo JA. 1952. Ecologia de la vizcacha (*Lagostomus maximus maximus*) en el nordeste de la provincia de Entre Rios. Rev Invest Agric 6:289.

Milloning GA. 1961. A modified procedure for lead staining of thin sections. J Biophys Biochem Cytol 11:736–739.

Mohamed F, Fogal T, Dominguez S, Scardapane L, Guzmán J, Piezzi RS. 1995. Ultrastructure of pars distalis of viscacha (*Lagostomus maximus maximus*): pituitary cellular types. Biocell 19:133–140.

Mohamed F, Fogal T, Dominguez S, Scardapane L, Guzmán J, Piezzi RS. 2000. Colloid accumulations in the pituitary pars distalis of viscacha (*Lagostomus maximus maximus*): a numeric study in relation to season, sex and growth. Anat Rec 258:252–261.

Morgan PJ, King TP, Lawson W, Slater D, Davidson G. 1991. Ultrastructure of melatonin-responsive cells in the ovine pars tuberalis. Cell Tissue Res 263:529–534.

Morgan PJ, Webster CA, Mercer JG, Ross AW, Hazlerigg DG, MacLean A, Barrett P. 1996. The ovine pars tuberalis secretes a factor(s) that regulates gene expression in both lactotropic and nonlactotropic pituitary cells. Endocrinology 137:4018–4026.

Morgan PJ. 2000. The pars tuberalis: the missing link in the photoperiodic regulation of prolactin secretion? J Neuroendocrinol 12:287–295.

Muñoz E, Fogal T, Domínguez S, Scardapane L, Guzmán J, Piezzi R. 1997. Seasonal changes of the Leydig cells of viscacha (*Lagostomus maximus maximus*): a ligth and electron microscopy study. Tissue Cell 29:119–128.

Oota Y, Kurosomi K. 1966. Electron microscopic studies on the pars tuberalis of the rat hypophysis. Arch Histol Jpn 27:501–520.

Rudolff T, Filler T, Wittkowski W. 1993. Pars tuberalis-specific cells within the pars distalis of the adenohypophysis: an ontogenetic study. Ann Anat 175:171–176.

Scardapane L, Lucero JB, Domínguez S, Piezzi RS, Guzmán JA. 1983. Effect of chronic administration of melatonin on vizcacha pars intermedia (*Lagostomus maximus maximus*). Commun Biol 2:183–188.

Scardapane L. 1990. Aspectos histofisiológicos de la pars intermedia hipofisiaria del *Lagostomus maximus maximus*: PhD thesis. San Luis, Argentina: Biblioteca Central Universidad Nacional de San Luis.

Stoeckel ME, Porte A. 1984. Ultrastructure and development of the pars tuberalis of the mammals. In: Motta PM, editor. Ultrastructure of endocrine cells and tissues. Boston: Nijhoff. p 29–38.

Stoeckel ME, Hindelang C, Klein MJ, Poissonnier M, Felix JM. 1994. Expression of the alpha-subunit of glycoprotein homones in the pars tuberalis-specific glandular cells in rat, mouse and guinea-pig. Cell Tissue Res 278:617–624.

Wittkowski W, Schulze-Bonhage A, Böckers T. 1992. The pars tuberalis of the hypophyis: a modulador of the pars distalis? Acta Endocrinol 126:285–290.

Wittkowski W, Bockman J, Kreutz MR, Böckers TM. 1999. Cell and molecular biology of the pars tuberalis of the pituitary. Int Rev Cytol 185:157–194.