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Pituitary Intermediate Lobe in Dog: Two Cell Types and High Bioactive Adrenocorticotropin Content

Abstract. *The pituitary intermediate lobe of most species is cytologically monotonous, but that of the dog is composed of two immunocytochemically distinct cell types. The predominant* A *cells are typical pars intermedia cells: they stain immunocytochemically for α-melanotropin and, more weakly, for adrenocorticotropin and β-lipotropin. The* B *cells are like the corticotrophs of the anterior lobe: they stain intensely for adrenocorticotropin and β-lipotropin but not for α-melanotropin. The* B *cells may account for the high concentration of bioactive adrenocorticotropin measured in the canine pars intermedia, and may explain why in dogs adenomas causing Cushing's disease through hypersecretion of adrenocorticotropin can arise from the intermediate as well as the anterior pituitary lobe.*

Recent studies have greatly clarified the biogenesis of adrenocorticotropin (ACTH). It is now evident that ACTH and β-lipotropin (β-LPH) are cleaved from a common precursor glycoprotein molecule (variously termed pro-ACTH/endorphin, pro-opiocortin, pro-corticomelanotropin, and pro-corticolipotropin) (*1*). In the corticotrophs of the hypophysial pars distalis (PD) the prohormone is predominantly processed to authentic ACTH [ACTH(1-39)], β-LPH, and variable amounts of γ-LPH and β-endorphin (*2*). In the pars intermedia (PI) of species thus far studied (rat, cow, and pig), ACTH(1-39) is *N*-acetylated and then cleaved into an NH_2-terminal tridecapeptide that, upon subsequent *C*-amidation, yields α-melanotropin (α-MSH); concomitantly, ACTH(18-39) (corticotropin-like intermediate lobe peptide, CLIP) is formed from the COOH-terminal portion of ACTH(1-39). β-LPH is also further processed in the PI to β-endorphin (the 31 COOH-terminal amino acids of β-LPH) and its metabolites (*3*). In the PI, therefore, ACTH and β-LPH appear to serve as intermediates in the biosynthetic pathway to α-MSH and β-endorphin, respectively. Since biological activity of ACTH, in a potency comparable to that of ACTH(1-39), minimally requires the sequence ACTH(1-18), it is not surprising that little bioactive ACTH has been found in the PI of rats (*4*, *5*). Inability of the rat PI to maintain the structure and function of the adrenal cortex after removal of the PD also indicates that the PI does not secrete physiologically significant amounts of bioactive ACTH (*6*).

It is of interest that Cushing's disease, that is, hypercortisolism due to excessive secretion of ACTH, can occur in dogs in association with tumors of the PI as well as the PD (*7*). Among 11 dogs with Cushing's disease associated with pituitary adenomas that we have thus far examined, eight had tumors of the PD and three of the PI. The fact that tumors secreting bioactive ACTH can arise from the canine PI suggested to us that the PI of this species may fundamentally differ from that of others such as the rat. We have therefore studied the canine PI (i) by immunocytochemistry (ICC) for ACTH and other derivatives of the prohormone molecule, (ii) by bioassay for ACTH, and (iii) by radioimmunoassay (RIA) for ACTH combined with physicochemical characterization of the reactive molecular species.

The dog hypophyses used were from 18 adult male and female German shepherds and Labrador retrievers, five of which were studied by ICC. For ICC comparison pituitaries were obtained from five rats, five mice, four cats, six rhesus monkeys, and nine human beings who had died from nonendocrine diseases. The antibodies used were the following. (i) Antibody to midportion ACTH (West) (supplied by the NIAMDD Hormone Distribution Program), which reacts with ACTH(1-39) and ACTH(11-24) on an equimolar basis, but not with α-MSH, β-MSH, ACTH(1-10), or ACTH(17-39). (ii) Antibody to human β-LPH whose antigenic determinant resides in the NH_2-terminal(1-36) sequence and which reacts with β-LPH and γ-LPH on an equimolar basis (*8*); although this antibody shows only partial cross-reaction with dog β-LPH, its cross-reactivity is sufficient for ICC. (iii) Antibody to α-MSH (supplied by H. Vaudry) that cross-reacts, when tested by RIA, less than 0.2 percent with human and porcine (p) ACTH(1-39), ACTH(1-10), and ACTH(1-16)-NH_2, and not at all with β-LPH or β-, γ-, or α-endorphin (*5*).

By ICC we found that in dogs, as in other species, the PD corticotrophs stained with the antibody to ACTH, and a very few of them stained with the antibody to α-MSH. In the canine PI we found two distinct cell types (Table 1): *A* cells, which comprised more than 90 percent of the cell population, and *B* cells,

Table 1. Immunostaining of the dog hypophysis for ACTH and its biosynthetic congeners. Glands were fixed in Bouin's fluid, embedded in paraffin, and sectioned at 5 μm. Pituitary peptides were demonstrated with the unlabeled antibody-peroxidase-antiperoxidase technique (*9*). The antibody dilutions used were 1:9000 for antibody to ACTH, 1:3000 for antibody to β-LPH, and 1:1000 for antibody to α-MSH. The antibodies were applied to the sections for 24 hours at 4°C, and the sites of antigen-antibody interaction demonstrated with 3,3'-diaminobenzidine (30 mg/ml) in the presence of 0.05 percent hydrogen peroxide as the chromogen. In control experiments we substituted normal rabbit serum for the immune serums, and absorbed the antibodies with the appropriate antigens (1 μg/ml) for 48 hours before applying them to the slides. Only staining abolished by such incubation of the antibody with the antigen was considered specific. The number of plus signs indicates the intensity of staining.

Cell type	ACTH	β-LPH*	α-MSH
Pars distalis corticotrophs	+++	+++	−−
Pars intermedia			
A cells	+	+	++
B cells	+++	+++	−−

*We have confirmed, in all species studied, that cells staining for ACTH also stain for β-LPH (*10*). No component of the dog hypophysis stained, at dilutions of 1:9000 and 1:3000, with an antibody against human β-endorphin that stained the PI of rats and the PD corticotrophs of cats reliably, PD corticotrophs of rats and mice erratically, and those of human beings not at all.

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Table 2. Comparison of bioactive and immunoreactive ACTH in the neurointermediate lobe (NIL) and pars distalis (PD) of dog and rat pituitaries. Animals were killed with excess pentobarbital and the exposed hypophyses separated into NIL and PD at the cranial end of the cleft between these lobes. The tissues were placed in 0.2*N* HCl containing 0.1 percent human serum albumin and homogenized. After centrifugation at 1400*g*, the supernatant was assayed for ACTH bioactivity in a dispersed adrenal cell system (*15*) and for ACTH immunoreactivity with the West antibody used at a final dilution of 1:15,000. Numbers in parentheses are 95 percent confidence limits.

Animal and tissue	Number of animals	Bioactive ACTH		Immunoreactive ACTH	
		Per lobe (μg)	Per milligram (ng)	Per lobe (μg)	Per milligram (ng)
Dog					
NIL	7	3.1 (2.3 to 3.8)	94 (67 to 122)	2.3 (1.4 to 3.2)	68 (43 to 94)
PD	7	15.9 (12.2 to 19.6)	169 (135 to 203)	39.4 (36.1 to 42.7)	424 (372 to 476)
Rat					
NIL	12	0.012 (0.009 to 0.1014)	8.0 (5.3 to 9.3)	0.07 (0.05 to 0.08)	47 (33 to 53)
PD	12	0.60 (0.51 to 0.69)	75.0 (64 to 86)	0.67 (0.60 to 0.74)	84 (75 to 93)

which were interspersed with the *A* cells throughout the PI (Fig. 1A). In some places where *B* cells formed clusters it was clear that they did not stain for α-MSH, although they were intensely reactive for ACTH (Fig. 1, B and C). The ICC thus revealed that only the *A* cells contained the characteristic PI hormone, α-MSH, and that staining of the *B* cells was the same as that of the PD corticotrophs.

The *A* cells of the canine PI resemble the PI cells of rats, mice, guinea pigs, hamsters, and monkeys, as described by others (*11*) and also observed by us. The postnatal hypophysis in humans lacks a distinct PI, but corticotrophs that may be present in the pars nervosa have been considered a functional equivalent of the PI (*12*) and stain variably with antibody to α-MSH (*13*). We have found cells staining for α-MSH in the rudimentary PI of some adult human hypophyses, and others (*14*), using ICC and ultrastructural criteria, have reported occasional cells resembling PD corticotrophs among typical PI cells in the rostral extremity of the PI of rodents.

In dogs, the concentration of bioactive ACTH in the neurointermediate lobe was half of that in the PD (Table 2); in rats, this concentration ratio was much lower, in agreement with several earlier reports (*4*, *5*). Since the ACTH in the neurointermediate lobe in rats is all located in the PI, and since the PI constitutes 50 percent of the neurointermediate lobe in the rat (*16*) and 25 percent in the dog, one can calculate that in the dog the concentration of bioactive ACTH in the PI is twice as high as in the PD and 24 times as high as in the rat PI. Further, in the dog, the concentration of bioactive ACTH in the neurointermediate lobe is about the same as that of immunoreactive ACTH, whereas in the rat the concentration of the latter is six times higher than that of bioactive ACTH.

Molecular sieve chromatography revealed that more than 80 percent of ACTH immunoreactive with the West antibody in extracts of canine neurointermediate lobe coeluted with pACTH(1-39) marker peptide. Cation exchange and reverse phase high-pressure liquid chromatographic analysis resolved this material into two components, one of which behaved like pACTH(1-39) and the other like its α-*N*-acetyl derivative (1.1 μg and 0.9 μg per lobe, respectively, in a pool of four neurointermediate lobes). Similar analysis of anterior lobes demonstrated that virtually all the bioactivity could be accounted for by material behaving like authentic pACTH(1-39). The additional immunoassayable ACTH (Table 2; note the discrepancy between immunoreactive and bioactive ACTH concentrations in the dog PD) was distributed among prohormone-like compounds, putative biosynthetic intermediates, and material less basic (and smaller) than pACTH(1-39).

The parenchyma of the PI in the dog, as in other species, consists entirely of cells genetically programmed to produce pro-corticolipotropin. The *A* and *B* cells apparently differ in their posttranslational processing of this prohormone (this difference may be secondary to differences in cleaving enzymes), but it cannot be assumed that this represents an irreversible differentiation. It is conceiv-

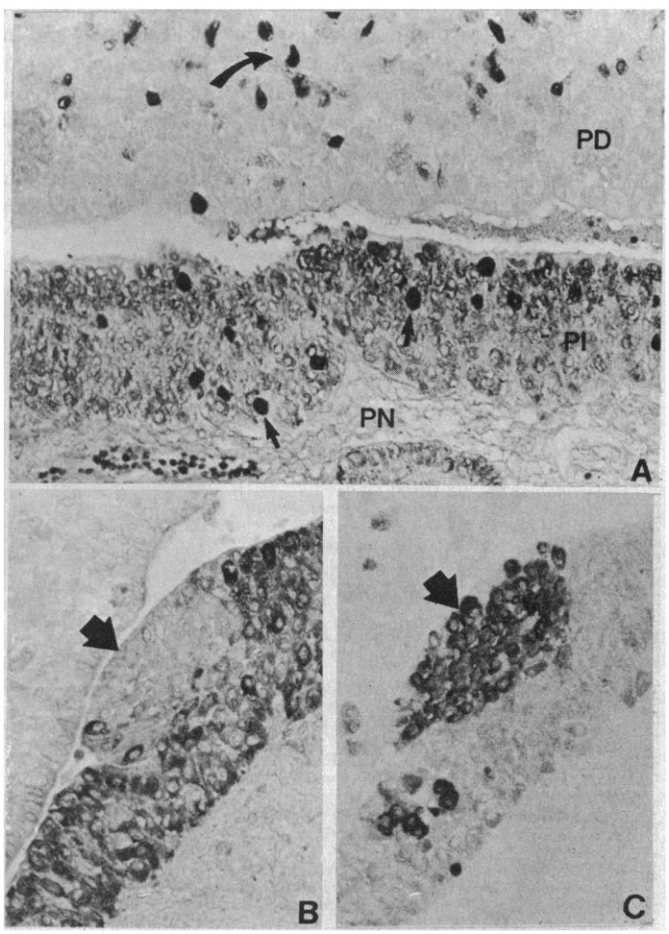

Fig. 1. (A) Pars intermedia (*PI*) of a dog hypophysis, immunostained for β-LPH. Note the dark *B* cells (arrows), which resemble the corticotrophs in the pars distalis (*PD*) (curved arrow). The predominant *A* cells in the PI are less intensely stained (*PN*, pars nervosa) (× 150). (B) A cluster of *B* cells in a PI stained for α-MSH (arrow) is unstained, in contrast with the surrounding *A* cells. (C) In an adjacent section stained for ACTH, the *B* cell cluster stands out owing to its staining that is much more intense than that of the *A* cells (×200).

able that *A* cells can be transmuted into *B* cells by loss of the enzymatic activities necessary for the formation of α-MSH. This type of change has been suggested for the PI cells of the trout, which in vitro appear to acquire the ability to secrete increasing amounts of bioactive ACTH while their α-MSH secretion decreases (although alternative explanations are possible) (*17*). Although the *B* cells resemble PD corticotrophs, it is possible that appropriate stimuli could cause them to cleave ACTH to α-MSH and CLIP, as *A* cells evidently do. Further, their functional regulation may differ from that of PD corticotrophs owing to their location in the avascular PI, which receives dopaminergic and serotonergic fibers from the brain (*18*), whereas the PD corticotrophs are devoid of a nerve supply and are controlled mainly by factors from the hypothalamus that reach them through the hypophysial portal vessels. Studies of the responses of separated *A* and *B* cells to various agents in vitro would therefore be of great interest.

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19. Porcine and ovine ACTH(1-39) were provided, respectively, by J. Ramachandran, University of California, and Ciba-Geigy. We thank A. E. Wallen, Sr., for his gift of the dogs. Supported by PHS grants AM-02463, NB-02893, and T32-AM-0727, Research Career Award AM-13,576 (to N.S.H.), National Research Service Award F-32-AM-06261 (to M.E.P.), and the Lita Annenberg Hazen Charitable Fund.

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Immunophagocytic Properties of Retinal Pigment Epithelium Cells

Abstract. *Retinal pigment cells were dislodged from normal monkey eyes and incubated in glass-slide chambers. All viable pigment cells adhered strongly to glass. They demonstrated surface receptors for the Fc portion of immunoglobulin G and for the third component of complement by selectively binding and phagocytizing antibody or complement-coated erythrocytes. These phagocytic cells with receptors were identified as retinal pigment cells by characteristic ultrastructural features. Thus, retinal pigment cells, which are generally believed to be derived from neural tissue, are not only scavengers of photoreceptor cell debris, but also have surface receptors and phagocytic functions that may be important in ocular defense.*

The retinal pigment epithelium (RPE) consists of a monolayer of cells interposed between the rod and cone photoreceptor elements of the sensory retina and the choroidal blood circulation. As a component of the blood-retina barrier (*1*, *2*), the neuroectoderm-derived (*3*, *4*) RPE participates in processes essential to photoreceptor cell homeostasis (*1*, *2*). Among the functions of the RPE is the phagocytosis and intracellular lysosomal degradation of aged photoreceptor membranes that are shed from the tips of rod and cone cells in a diurnal cycle (*5–7*). The phagocytic nature of the RPE (*8*, *9*) led us to investigate whether specific receptor mechanisms known to mediate the attachment, ingestion, and elimination of particulate matter by other phagocytes might also be demonstrable in RPE cells. In particular, glass-adherent macrophages of bone marrow origin have surface receptors for the Fc region of immunoglobulin G (IgG) antibody and for the third component of complement, both of which bind and thereby promote the phagocytosis of specifically coated particles (*10*, *11*). We therefore used erythrocytes coated with IgG and complement-coated erythrocytes to demonstrate receptor-mediated binding and phagocytosis by RPE cells.

Suspensions of retinal pigment cells were prepared from *Macaca fasicularis*

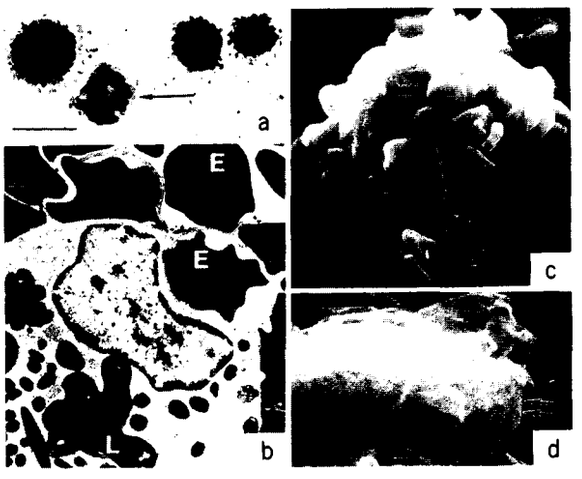

Fig. 1 (a) Photomicrograph of EA rosettes. Deeply pigmented RPE cells obtained from *M. fasicularis* have bound human erythrocytes coated with IgG antibodies to red blood cells. In one cell (arrow), pigment granules appear to be displaced by phagocytized erythrocytes. Scale bar, 50 μm. (b) Transmission electron micrograph of a portion of a RPE cell from *M. fasicularis*. The pigment cell has bound IgG-coated erythrocytes (*E*). Tight attachment has caused many of them to become deformed. Two erythrocytes are apparently being engulfed by the RPE cell which exhibits typical features such as round and elliptical melanin granules, combined bodies, and a lamellar figure (*L*) believed to represent rod or cone (or both) membrane stacks within a secondary lysosome. Scale bar, 2 μm. (c) Scanning electron micrograph of a RPE cell EA rosette. Many of the IgG-sensitized red blood cells bound to this *M. fasicularis* RPE cell are being engulfed by membrane ruffles. A recently phagocytized erythrocyte may be seen just beneath the cell surface (arrow). Scale bar, 5 μm. (d) Higher magnification of a sensitized erythrocyte (arrows) undergoing phagocytosis by a RPE cell. Scale bar, 2 μm.

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