THE PRESENCE OF SUPPORTIVE CELLS IN THE PRENATAL DEVELOPMENT OF SHEEP PINEAL GLAND

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Free cell types have been defined in mammal pineal gland parenchyma: pinealocytes or principal cells, supportive or interstial cells and pigmented cells. Numerous terms have been used to designate the second type, including interstitial cells, type II pinealocytes, glial cells and astrocytes.

Ultrastructural and immunohistochemical techniques were used to study the second cell type in sheep embryo pineal glands. 32 embryos were studied from day 54 of development through birth. Specimens were arranged in four age-groups, defined in terms of the most relevant histological features: group 1 (54 to 67 days of prenatal development), group 2 (71 to 92 days), group 3 (98 to 113 days) and group 4 (118 to 150 days).

Embryo pineal glands were sliced parasagitally after 1 hour in Carnoy's fluid. One of the two portions thus obtained was fixed in 10% neutral formalin saline and processed by paraffin-embedding methods. Sections 3 um thick were cut and stained with hematoxylin and eosin (HE) for routine morphological examination, and with phosphotungstic acid hematoxylin (PTAH) for detection of glial-type cells.

An avidin-biotin-peroxidase complex (ABPC) was carried out on deparaffinized pineal samples for detection of glial fibrillary acidic protein (GFAP), the main protein component of intermediate astrocyte filaments.

The other half of each pineal gland was used for ultrastructural analysis. Tissue blocks (1 mm^3) were immersed in ice-cold 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2), and embedded in epoxy resin. Ultrathin sections cut from blocks obtained for electron microscopy were stained with colloidal gold (10 nm) for detection of GFAP positive cells.

The pineal parenchyma in groups 1 and 2 displayed a uniform morphology; cells were all of one type, identified as pinealoblasts. In groups 3 and 4, in addition to pinealoblasts, a second cell type with different characteristics was detected. Cell morphology was similar in both these groups, and cells were generally located in the perivascular space. Nuclei were small, oval or rounded, and electron-dense, and thus readily distinguishable from the vesicular nuclei of pinealoblasts.

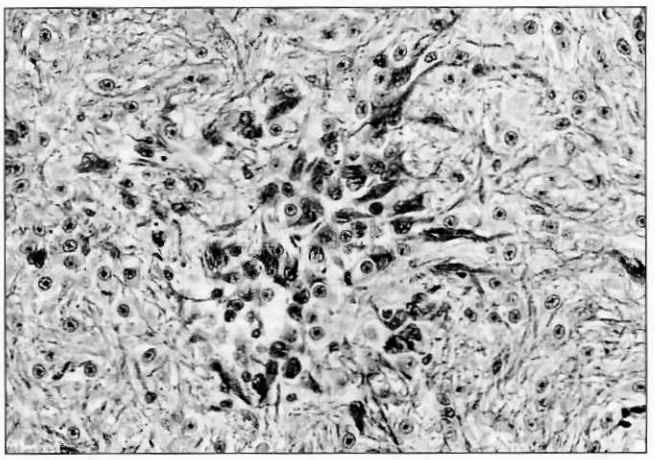

Figure 1. Embryo, 98 days. Positive staining cells (GFAP +) scattered throughout the pineal gland. ABPC x 350

Ultrastructural analysis confirmed light microscopic findings: a second cell type was evident in groups 3 and 4, in addition to the pinealoblasts present in all groups. Type II cells were less numerous, less electron-dense and showed a clear preference for perivascular locations . In group 3 these cells had oval nuclei, with a slender rim of chromatin bordering the nuclear envelope. The electron-lucent nucleoplasm, frequently contained a nucleolus of clearly-defined nucleolemma. The most characteristic feature of these cells was the presence of very long processes with numerous microfilaments. The endoplasmic reticulum was mostly granular; cisternae had fairly narrow lumina. Lysosomes with clearly-defined limiting membrane, diplosomes and ribosomes were observed in perinuclear cytoplasm .

Ultrastructurally, type II cells in group 4 closely resembled those observed in group 3, except that the former exhibited a greater number of filaments. Filaments showed a clear tendency to fuse with pinealoblast processes. A further differential characteristic was that type II cells in group 4 were more electron dense than those of group 3, due in part to the abundance of ribosomes, small electron-dense mitochondria, microfilaments and condensed chromatin.

GFAP positive cells were observed in the embryonic pineal parenchyma in groups 3 and 4. In group 3 these were distributed uniformly throughout the gland (Fig. 1). Immunopositive cells (whose appearance resembled that of the CNS astrocytes used as positive control) displayed small, dense, ovoid nuclei, and an intensely-staining rim of cytoplasm bordering negative nuclei. GFAP positive cells displayed a small number of processes, with varying diameters and arranged both longitudinally and transversally Cell processes were interwoven amongst pinealoblasts, and around blood vessels to form a limiting barrier.

In group 4 embryos, GFAP positive cells were either oval or elongated in shape. Cytoplasmic processes, which were more numerous than in group 3, varied in both diameter and orientation.

In both groups, colloidal gold labeling revealed expression of GFAP by cells whose morphology closely resembled that described ultrastructurally for the second cell type. These cells were observed in perivascular locations, exhibited ovoid and/or elongated non-staining nuclei, and strong cytoplasmic positivity, with clear affinity for the microfilaments of cytoplasmic processes (Fig. 2). Immunostaining was more intense in group 4 than in group 3 embryos. Adjacent pinealoblasts remained immunonegative.

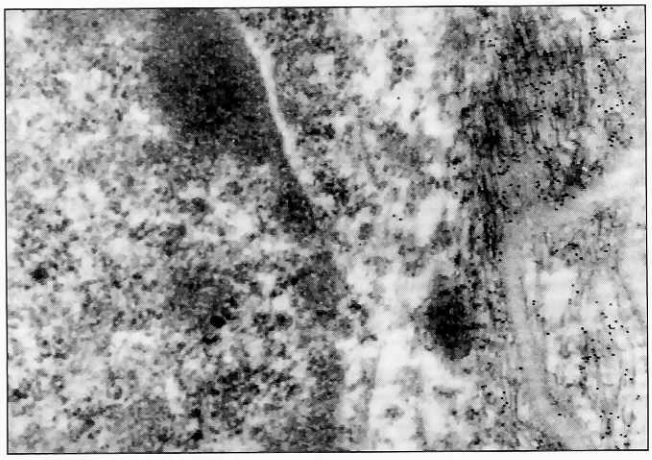

Figure 2. Embryo, 113 days.Inmune electronmicroscopic demostration of GFAP in the cytoplasm of type II cells. IEM x 20.000.

The results obtained indicate the existence of a second cell type, in addition to pinealoblasts, during the prenatal development of sheep pineal gland. Light microscopy highlighted the resemblance between these cells and the second cell type described in the pineal gland of other species. However, ultrastructural homogeneity rendered impossible the detection of possible subtypes. Histochemical and immunological tests were thus performed with a view to overcoming this limitation and at the same time enhancing our poor knowledge of this second cell type. The first step was to determine the presence of glial-like cells by PTAH immunostaining.

GFAP is widely considered a valid label for the detection of astrocyte development and more particularly as evidence of the presence of astrocytic cells at a certain stage of maturity (López-Muñoz et al. 1992). The second step was thus to determine GFAP expression.

Comparison of the results of these two tests (PTAH and GFAP) showed that of all glial (PTAH positive cells), only a proportion were positive to GFAP. The numerical density of GFAP positive cells was lower than that of PTAH positive cells, suggesting that a certain proportion of glial (PTAH positive) cells may be immature astrocytes not expressing GFAP, a finding already reported in rats (Schachner et al. 1984) and in carnivores (López-Muñoz et al. 1992; Boya and Calvo 1993).

GFAP positive cells were observed in the embryonic pineal parenchyma in group 3 and 4. In group 3 these were distributed uniformly throughout the glandThe results obtained here indicate that the second cell population in developing ovine pineal gland is in fact a combination of glial-astrocyte cells at varying stages of maturity. This hypothesis hasalready been advanced in the case of adult rat pineal gland (López-Muñoz et al. 1992).

Because the second cell type was detected here at 98 days gestation, sheep can be classed as a species showing early astrocyte maturation. Comparison of the location of type II cells in various mammalian species suggests that cell topography is closely linked to the anatomical location of the gland. In sheep, where the gland is deeply located within nerve structures, GFAP positive cells are detected across the whole gland surface. Similar results have been reported in hamsters (Sheridan and Reiter 1970), and in cats and dogs (Boya and Calvo 1993). In rats, however, where the pineal gland is more superficially located, these cells are found only in the stalk and the most superficial portion of the gland (López-Muñoz et al. 1992).

In the course of ovine embryonic development the vascular affinity of this second cell population, composed of glial-like or astrocytic cells at varying stages of maturity, leads to the formation of a blood pineal barrier. This barrier may constitute the morphological expression of a possible functional involvement in the exchange of substances between blood and pineal parenchyma.

References

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