

Immunohistochemical Studies on Supporting Cells in the Adrenal Medulla and Pineal Gland of Adult Rat, Especially on S-100 Protein, Glial Fibrillary Acidic Protein and Vimentin

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Abstract: In the adrenal medulla and pineal gland, the morphological and chemical nature of supporting cells were examined immunohistochemically. In the adrenal medulla, supporting cells in noradrenaline (NA)-cell regions showed similar and intense immunoreactivities to the three glial marker proteins, S-100 protein, glial fibrillary acidic protein (GFAP) and vimentin, forming a network, while those in adrenaline (A)-cell regions were less numerous and their immunoreactivity was positive to S-100 protein but negative to GFAP and, at least in part, negative to vimentin. In the pineal gland, supporting cells in the stalk and the proximal region of the body portion formed a network and showed immunoreactivities to the three antibodies, while those in the distal region of the body portion were less numerous and their immunoreactivity was positive to S-100 protein and vimentin, but negative to GFAP. Thus, the distribution pattern and chemical nature of supporting cells showed regional differences in both glands. There were several similarities in supporting cells between those in NA-cell regions of the adrenal medulla and in the stalk and the proximal region of the body portion of the pineal gland, and also between in A-cell regions of the adrenal medulla and in the distal region of the body portion of the pineal gland, respectively. The biological and functional significances of these results are discussed.

Key words: adrenal medulla, pineal gland, supporting cells, glial marker protein, rat

Introduction

The adrenal medulla and pineal gland are the endocrine organs which originate from the nervous tissue. In the rat, both glands contain at least two types of parenchymal cells, that is, the chief, amine-secreting type of cells (chromaffin cells and pinealocytes) and the other type of cells (sustentacular (Böck, 1982) or supporting cells (Coupland, 1984) in the adrenal medulla and glial cells in the pineal gland (Vollrath, 1981; Pévet, 1983)). However, since the neuroglial cells are classified as supporting cells in the central nervous system (Peters et al., 1991), we designate the latter type of cells as supporting cells in this paper.

S-100 protein and two different types of intermediate filament proteins, i.e., glial fibrillary acidic protein (GFAP) and vimentin (VIM), are now known as glial marker proteins. In support-

ing cells of the adrenal medulla, S-100 protein has been reported to be present (Cocchia and Michetti, 1981; Stefansson et al., 1982; Iwanaga and Fujita, 1984; Lauriola et al., 1985; Lloyd et al., 1985; Suzuki and Kachi, 1993, 1994), but no literature is available on the presence of GFAP and VIM. On the other hand, the presence of these three glial marker proteins have been shown in supporting cells of the pineal gland (Møller et al., 1978; Huang et al., 1984; Schachner et al., 1984; Zang et al., 1985; Calvo et al., 1988; Kachi et al., 1989; Yamamoto et al., 1990; López-Muñoz et al., 1992; Borregón et al., 1993; Boya and Calvo, 1993).

We previously reported that the extent of cellular association of chromaffin cells with supporting cells was remarkably higher in noradrenaline-chromaffin (NA) cell regions than in adrenaline-chromaffin (A) cell regions in the adrenal medullae of the rat and pig, by quantitative electron microscopy (Kachi et al., 1993) and immunohisto-

chemistry using antibody to S-100 protein (Suzuki and Kachi, 1994).

In the present study, we examined the adrenal medulla and pineal gland of the rat by an immunohistochemical method using antisera to S-100 protein, GFAP and VIM, and whether there are regional differences not only in the morphology but also in the chemical nature of supporting cells in these glands, and whether there are any relationships in such regional differences between these two glands.

Materials and Methods

Nine male Wistar rats at 10 weeks of age were used in this study. Animals were maintained in a windowless animal room with controlled temperature ($22\pm 2^\circ\text{C}$) and light (LD 12:12) with free access to food and water. Rats were killed by decapitation, and the adrenal gland and pineal gland with the surrounding brain tissues were removed immediately, the former cut into halves, and fixed with Zamboni fluid for 24 h at room temperature. After being rinsed overnight in 0.1 M phosphate buffer (pH 7.4) containing 5% (w/v) sucrose, these blocks were immersed in 30% sucrose in phosphate buffer for 12 h at 4°C . The blocks were embedded in O.C.T. compound (Miles Scientific Co., USA) and frozen rapidly in liquid nitrogen. The frozen blocks were cut into serial sections $10\ \mu\text{m}$ in thickness. The pineal blocks were cut sagittally with a cryostat and mounted on poly-L-lysine-coated glass slides.

Immunohistochemistry

Sections were stained immunohistochemically using antisera to phenylethanolamine *N*-methyltransferase (PNMT) (Eugene Tech International, USA), S-100 protein (DAKO Co., USA) and GFAP (Zymed Lab. Inc., USA), and by the avidin-biotin peroxidase complex (ABC) method (Hsu et al., 1981), using a commercially available ABC kit (Vector Lab. Inc., USA). A labelled streptavidin biotin (LSAB) method (LSAB kit; DAKO Co., USA) was used for VIM (DAKO Co., USA). Primary antibodies were diluted at 1:1,000 for PNMT and S-100 protein, 1:100 for GFAP and 1:25 for VIM, respectively. Immunohistochemical procedures were carried out as described previously (Suzuki

and Kachi, 1994). Following immunohistochemical staining, the sections were counterstained lightly with 1% methyl green and examined with a light microscope.

Results

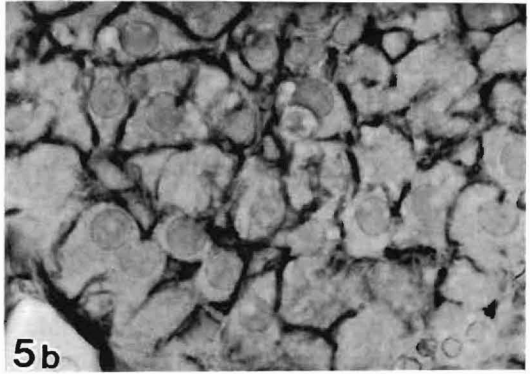
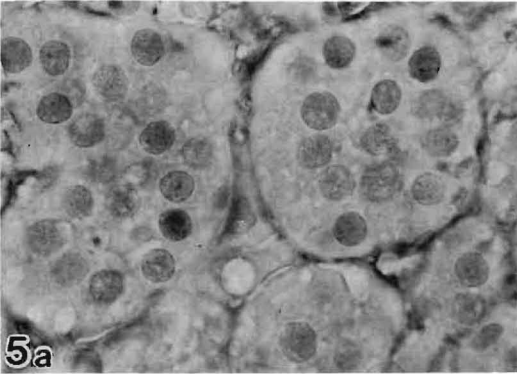
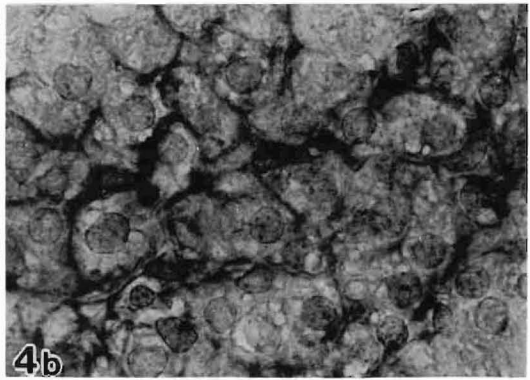
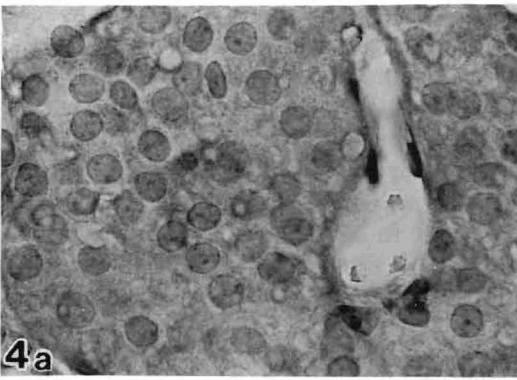
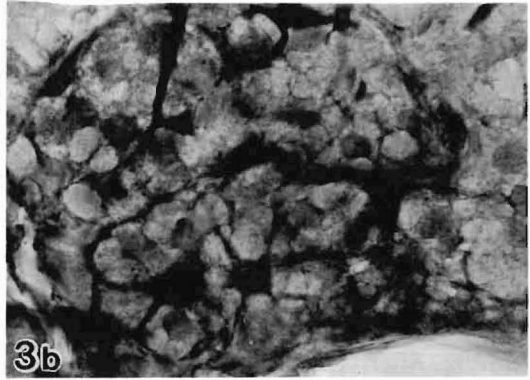
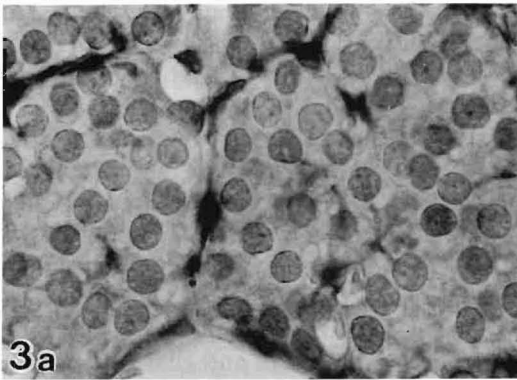
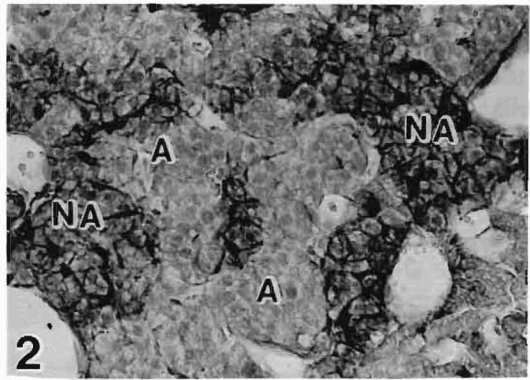
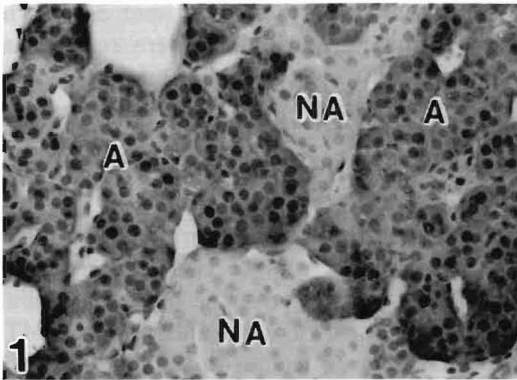
The following findings were obtained by careful examination of sets of two or three adjacent sections. One section of the adrenal medulla was immunostained using the antibody to PNMT and others using antibodies to the glial marker proteins. The pineal sections were stained using antibodies to the same glial marker proteins.

Adrenal medulla

Anti-PNMT. PNMT-immunoreactive cells (A cells) occupied large areas in the adrenal medulla, and small islets of PNMT-negative cells (NA cells) were scattered among those A-cell regions (Fig. 1). PNMT-immunoreactivity was not found in any of the adrenocortical cells.

Anti-glial marker proteins. The immunohistochemical reactions to anti-glial marker proteins were negative in adrenocortical cells and adrenomedullary chromaffin cells.

S-100 protein: Intensely S-100-positive cells were scattered among chromaffin cells in the adrenal medulla. The occurrence of immunoreactive (supporting) cells was apparently more frequent in NA-cell regions than in A-cell regions (Fig. 2). In NA-cell regions, many S-100-labelled cells were observed in the internal portion of the parenchyma, extending lamellar cytoplasmic processes radially between NA cells. A few S-100-positive cells were also seen in the marginal portion, having thin cytoplasm and extending cytoplasmic processes between NA cells and the interstitial tissue and incidentally between NA cells. These numerous processes of immunoreactive supporting cells took part in the formation of mesh-work in each NA-cell island (Figs. 2 and 3b). In A-cell regions, only a few S-100-positive cell bodies and/or processes were seen in the marginal and/or internal portions of the parenchyma (Fig. 3a). In both regions, S-100-positive cells located in the internal portion of the parenchyma were star-shaped, and those located in the marginal portion did not show radial projections in profiles.



GFAP: In NA-cell regions, the distribution pattern and form of GFAP-positive cells were similar to those of S-100-labelled cells, while intracellular immunoreactive profiles were somewhat more delicate, loose or slender in both the perinuclear cytoplasm and processes compared with those in S-100-positive cells (Fig. 4b). In A-cell regions, GFAP-immunoreactive cells and/or their cytoplasmic processes were scarcely found (Fig. 4a).

VIM: VIM immunoreactivity was observed in endothelial cells of blood vessels, fibroblasts and Schwann cells in the interstitial tissue of both adrenal cortex and medulla, and also in supporting cells in the parenchyma of the adrenal medulla. In NA-cell regions, VIM-positive supporting cells and their cytoplasmic projections were seen chiefly in the internal portion of the parenchyma, showing a net-like arrangement (Fig. 5b). Such a distribution pattern was analogous to those of S-100- and GFAP-positive supporting cells. Intracellular immunoreactive profiles were generally smaller or more slender in VIM as well as GFAP than in S-100 protein, but slightly denser in VIM than in GFAP in the perinuclear cytoplasm. A-cell regions and/or their vicinities also showed the VIM immunoreactivity whose location was in the marginal portion of the parenchyma or in the interstitial tissue surrounding the parenchyma (Fig. 5a), but could not be determined exactly at the light microscopic level.

Pineal gland

No pinealocytes in any regions were immuno-

reactive for the three glial marker proteins. Supporting cells showed different expression of the marker proteins depending on their location.

S-100 protein: Star-shaped cells positively immunostained with anti-S-100 protein were numerous and densely distributed especially in the stalk and the proximal region of the body portion of the pineal gland (Fig. 6a). These cells sent out their cytoplasmic processes forming a network among pinealocytes (Fig. 7b). In the distal region of the body portion of the pineal gland, S-100-positive cell bodies and their radially distended cytoplasmic processes were seen among pinealocytes, but less numerous than in the more proximal regions (Fig. 7a).

GFAP: In the stalk and the proximal region of the body portion of the pineal, GFAP immunoreactive cells showed similar morphological characteristics to those of S-100-reactive cells (Figs. 6b and 8b), but in the distal region of the body portion, GFAP-positive cells were not seen (Figs. 6b and 8a).

VIM: VIM-positive immunoreactivity was found in supporting cells and endothelial cells of blood vessels. VIM-positive supporting cells and cytoplasmic processes were dispersed throughout the gland (Figs. 6c and 9a, b). However, the network formed by immunoreactive elements was somewhat looser in VIM than in S-100 and GFAP, in the stalk and the proximal region of the body portion of the pineal gland (Fig. 9a).

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- Fig. 1. PNMT immunoreactivity in the adrenal medulla. Positively stained cells are A cells (A), and NA cells (NA) are PNMT-negative. $\times 200$.
- Fig. 2. S-100 protein immunoreactivity in the adrenal medulla. S-100-positive cells are present more frequently in NA-cell regions (NA) than in A-cell regions (A), determined by comparing with the adjacent section immunostained with anti-PNMT. $\times 200$.
- Fig. 3. S-100 protein immunoreactivity in A-cell regions (a) and in NA-cell regions (b). In A-cell regions, a few S-100-positive cells are seen in the parenchyma. In NA-cell regions, many S-100-labelled cells and cytoplasmic processes are present in the parenchyma, forming a network among NA cells. $\times 640$.
- Fig. 4. GFAP immunoreactivity in A-cell regions (a) and in NA-cell regions (b). GFAP immunoreactivity is negative in supporting cells in A-cell regions. In NA-cell regions, the morphological features of GFAP-positive cells are similar to those of S-100-positive cells. $\times 640$.
- Fig. 5. VIM immunoreactivity in A-cell regions (a) and in NA-cell regions (b). In A-cell regions, VIM-positive immunoreactivity is seen in the marginal portion of the parenchyma and/or their vicinities. In NA-cell regions, the distribution pattern and form of VIM-positive cells are almost analogous to those of S-100- (Fig. 3b) and GFAP-positive cells (Fig. 4b). $\times 640$.

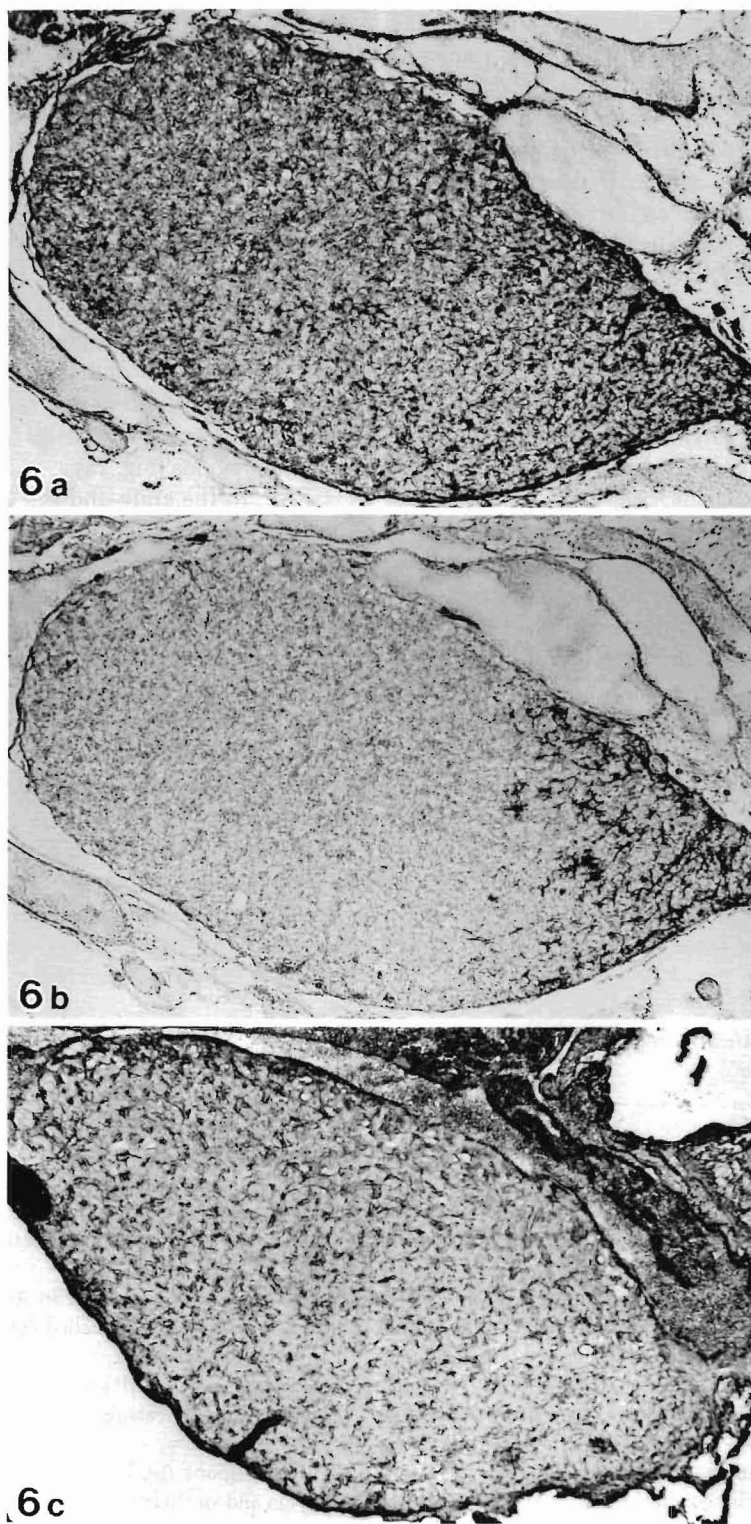
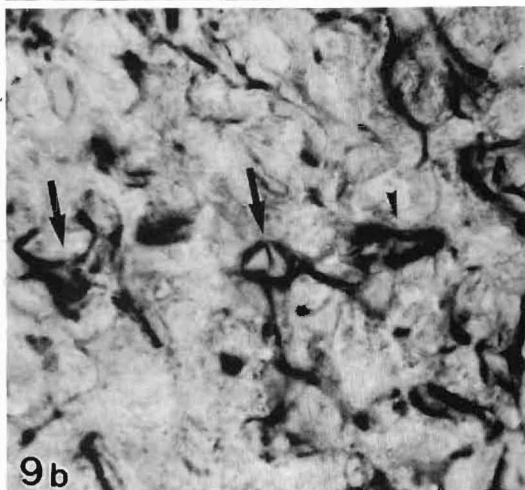
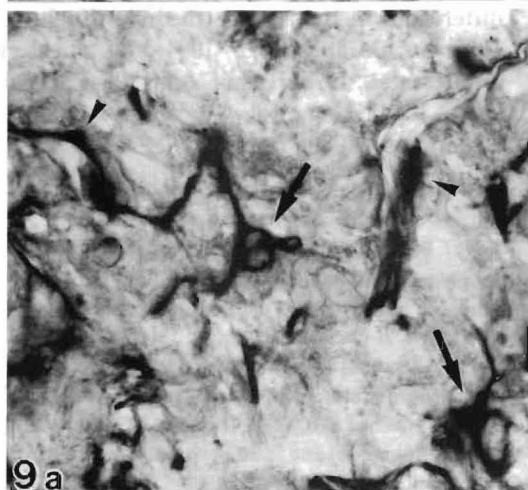
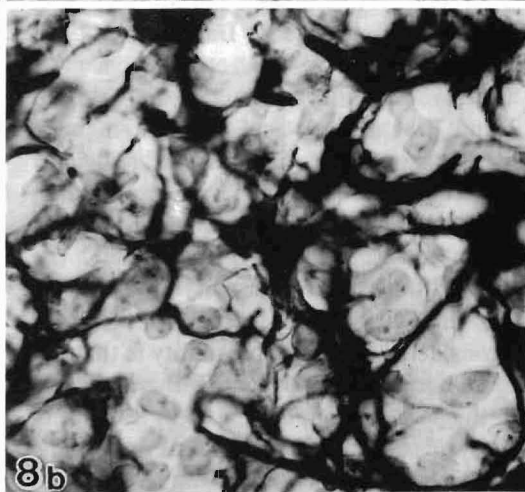
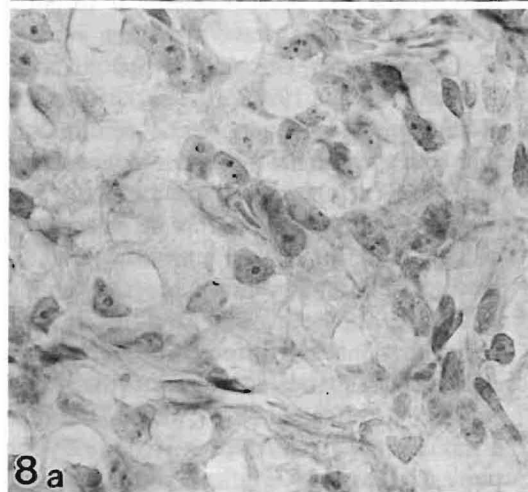
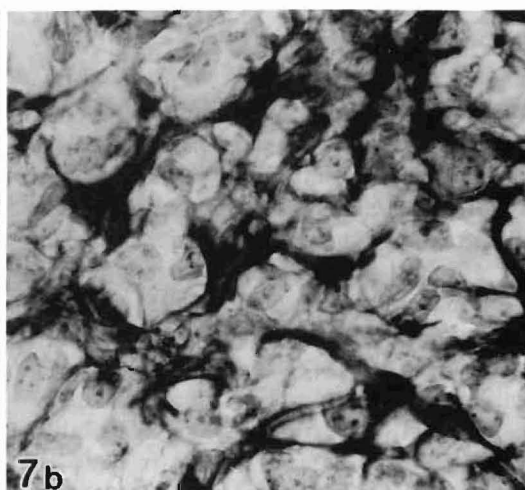


Fig. 6. Immunohistochemical staining for S-100 protein (a), GFAP (b), and VIM (c) in the pineal gland. In the stalk and the proximal region of body portion, supporting cells show immunoreactivities to three marker proteins. In the distal region, immunoreactivity is positive to S-100 protein (a) and VIM (c), but negative to GFAP (b). GFAP immunoreactivity is restricted to the stalk and the proximal region of body portion (b). a, b: $\times 48$, c: $\times 56$.



Discussion

S-100 protein is the cytoplasmic calcium-binding protein (Moore, 1965), and GFAP and VIM are two different types of intermediate filament proteins which are included in the glial filaments. GFAP-containing filaments are present in mature astrocytes of the central nervous system (CNS), and VIM filaments are present in immature astrocytes of the CNS and distribute extensively among different types of cells. Although the GFAP was initially considered to be present exclusively in the CNS, it has recently been shown that GFAP-like peptides are also present in the peripheral nervous system (Jessen and Mirsky, 1980, 1983; Yen and Fields, 1981; Dahl et al., 1982; Kobayashi et al., 1986; Mokuno et al., 1989).

In the present study, immunoreactivities to GFAP and VIM as well as S-100 protein were expressed in supporting cells in NA-cell regions of the adult rat adrenal medulla, while supporting cells in A-cell regions were immunopositive to S-100 protein but negative to GFAP. Regarding the location of VIM immunoreactivity in A-cell regions and their vicinities, at least two possibilities can be considered. The first possibility is that the VIM immunoreactivity is negative in supporting cells in the parenchyma, but exists in fibroblasts and Schwann cells in their interstitial tissue. The second possibility is that supporting cells consist of at least two types of cells, one type is located in the internal portion of the parenchyma which is VIM-negative and the other type is located in the marginal portion which is VIM-positive only in the processes between A cells and the interstitial tissue. More detailed analysis is needed to deter-

mine the more precise location.

In the pineal gland, it has been reported that S-100 protein and GFAP immunoreactivities are restricted to supporting cells located in the stalk and the proximal region of the body portion, and VIM-positive supporting cells are dispersed throughout the gland (Møller et al., 1978; Huang et al., 1984; Schachner et al., 1984; Zang et al., 1985; Calvo et al., 1988; Yamamoto et al., 1990; López-Muñoz et al., 1992; Borregón et al., 1993). Our present results coincide essentially with those of previous reports, except that S-100-positive cells exist in the distal region of body portion of the pineal, as shown by Yamamoto et al. (1990). The cells exhibiting immunoreactivity for calcium-binding spot 35 protein, identical to S-100-immunoreactive cells, have also been reported to distribute evenly throughout the pineal organ (Yamamoto et al., 1990).

Thus, previous and present results indicate that both the adrenal medulla and pineal gland in the adult rat show regional differences in the degree of cellular association of chief endocrine cells (chromaffin cells or pinealocytes) with supporting cells and in the expression of marker proteins in supporting cells, and that at least two subpopulations are differentiated in supporting cells in both glands according to their different expressions of the marker proteins, most clearly demonstrated as GFAP-positive and GFAP-negative cells. Possible significances of these regional differences in the adrenal medulla (Kachi et al., 1993; Suzuki and Kachi, 1994) and pineal gland (Møller et al., 1978; Huang et al., 1984; Schachner et al., 1984; Zang et al., 1985; Calvo et al., 1988; Kachi et al., 1989; Yamamoto et al., 1990; López-Muñoz et al., 1992; Borregón et al., 1993) have

Fig. 7. S-100-positive supporting cells located in the distal region (a) and in the proximal region (b) of the body portion in the pineal gland. In the proximal region, immunoreactive cells extend cytoplasmic processes radially and form a network among pinealocytes (b). $\times 640$.

Fig. 8. GFAP immunoreactivity in the distal region (a) and in the proximal region (b) of the body portion. GFAP-positive immunoreactive supporting cells are not present in the distal region. In the proximal region, the distribution pattern and form of GFAP-positive cells are similar to those of S-100-labelled cells. $\times 640$.

Fig. 9. VIM-positive supporting cells (arrows) located in the distal region (a) and in the proximal region (b) of the body portion. The morphological features of VIM-labelled supporting cells in both regions are essentially similar. In the proximal region, the network formed by VIM immunoreactivity in supporting cells is somewhat looser compared with those formed by S-100 and GFAP immunoreactivities. Endothelial cells (arrowheads) of blood vessels are also immunopositive. $\times 640$.

already been discussed. Supporting cells located in NA-cell regions of the adrenal medulla and in the stalk and the proximal region of body portion of the pineal coexpress two types of intermediate filament proteins, GFAP and VIM. The expression of intermediate filament proteins in astrocytes in the CNS has been reported to change during development from [GFAP-negative, VIM-positive], via [GFAP-positive, VIM-positive], to [GFAP-positive, VIM-negative] (Pixley and De Vellis, 1984). It seems to be significant to explore the changes in the expression of glial marker proteins in supporting cells occurring in the developing pineal gland (Borregón et al., 1993; Boya and Calvo, 1993; Kaneko et al., 1993). However, the coexpression of GFAP and VIM has also been demonstrated in astrocytes in nerve tissues, e.g., optic nerve (Calvo et al., 1990) and cerebellum (Schnitzer et al., 1981), of adult animals, and in supporting cells dispersed throughout the pineal gland of adult animals having deeply placed pineals (Boya and Calvo, 1993). The coexpression has been also observed in reactive astrocytes around lesions (Schiffer et al., 1986; Takamiya et al., 1988). Therefore, it may not be simply ascribed to immaturity of supporting cells, but it may have some relevance, at least in part, to adaptation to their local environment including functional differentiation.

Comparing the morphological feature and chemical nature of supporting cells in the adrenal medulla and pineal gland, it is apparent that there

are several similarities in supporting cells between in NA-cell regions of the adrenal medulla and in the stalk and the proximal region of the body portion of the pineal gland, and also between those in A-cell regions of the adrenal medulla and in the distal region of the body portion of the pineal gland, respectively, although differences were also seen in terms of the form of, and the VIM expression in, supporting cells between in the latter set of corresponding regions of the two organs. From these findings, interesting questions emerge whether a common, unknown factor(s) from NA-cell regions in the adrenal medulla and the proximal portion of the pineal gland stimulates the growth and differentiation of supporting cells, and whether these two sets of corresponding regions in the two organs have related functions or biological characteristics, respectively. At least, the nature of the former regions in both organs seems to be closer to that of nervous tissue than the latter regions which seem to be more typical endocrine tissues (Pévet, 1983; Coupland, 1984; Korf et al., 1989; Chen et al., 1989; Kachi et al., 1993). However, the biological and functional significances of the present results mostly remain to be clarified.

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成熟ラット副腎髄質と松果体における支持細胞の免疫組織化学的研究

—特に S-100 蛋白, GFAP, ビメンチンについて—

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神経組織に由来する内分泌腺である副腎髄質と松果体において、支持細胞の形態学および化学的性質を免疫組織化学的に検索し、比較・検討した。

12 時間毎の明暗周期、 $22 \pm 2^\circ\text{C}$ の環境下で飼育した生後 10 週の雄性ウィスター系ラット 9 匹を用い、副腎髄質および松果体を Zamboni 液で固定後厚さ 6~10 μm の凍結切片を作製、3 種のグリア細胞標識抗原に対する抗体、すなわち、抗 S-100 蛋白抗体、抗 GFAP 抗体、抗ビメンチン抗体を用い、ABC 法または LSAB 法により検索した。アドレナリン (A) 細胞は抗 A 合成酵素 (PNMT) 抗体により同定した。

副腎髄質：副腎髄質を構成する A 細胞領域とノルアドレナリン (NA) 細胞領域を比較すると、3 種のグリア性抗体に対する陽性支持細胞は、いずれも後者において前者よりも密に存在した。NA 細胞領域では、3 種の陽性支持細胞はほぼ同様の分布様式を示し、その核周部は実質辺縁・内両部に見られた。辺縁部支持細胞の胞体と板状突起は NA 細胞集団を被覆するとともに NA 細胞間にも入り込み、また、内部支持細胞の胞体突起は放射状に NA 細胞間に入り込んで全体として網工を形成していた。A 細胞領域では、S-100 蛋白陽性細胞が主として実質辺縁部にわずかに認められたが、GFAP 陽性細胞はほとんど認められなかった。ま

た、ビメンチン陽性反応が実質辺縁部付近に認められたが、間質の細胞との識別には今後の検討を要する。

松果体：S-100 蛋白と GFAP 陽性支持細胞は、松果体柄から体部近位側にかけてより密に存在し網工を形成していた。体部遠位側では松果体細胞間に放射状に胞体突起を伸ばす S-100 蛋白陽性細胞が散在性に認められたが、GFAP 陽性細胞は認められなかった。ビメンチン陽性細胞は松果体全域に見られたが、ビメンチン陽性反応による網工は一般に疎で、柄から体部近位側にかけても S-100 蛋白や GFAP 陽性反応に見られるような密な網工は観察されなかった。

結論として、ラット副腎髄質と松果体において、主要な腺細胞 (クロム親性細胞、松果体細胞) と支持細胞との細胞性連合の程度に器官内不均一性が認められ、両器官の実質を構成する支持細胞には少なくとも 2 型が区別された。また、副腎髄質 NA 細胞領域と松果体柄から体部近位側、さらに副腎髄質 A 細胞領域と松果体体部遠位側の各々に分布する支持細胞間に、形態学的ならびに化学的性質におけるいくつかの類似性が、特に前 2 者に、顕著に認められた。

Key words: 副腎髄質, 松果体, 支持細胞, グリア細胞標識蛋白, ラット