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Quantitative and immunohistochemical studies on supporting cells in the rat and pig adrenal medullae

Takao Suzuki [1] and Takashi Kachi [2]

Abstract

The number and chemical nature of supporting cells were examined in the adrenal medulla of the pig and rat. Cryostat sections of adrenal medullae fixed with Zamboni fluid were immunostained by the ABC method using antisera to PNMT, S-100 protein and GFAP. The relative number of nonchromaffin cells to total intraparenchymal cells was higher in NA-cell regions (pig: 22.3 ± 1.3%; rat: 20.2 ± 0.7%) than in A-cell regions (9.5 ± 0.7%; 7.9 ± 0.5%) in both species. In the pig adrenal medulla, the numerical ratio of S-100 protein-immunopositive cells to total nonchromaffin cells was higher in NA-cell regions (91.0 ± 1.9%) than in A-cell regions (29.9 ± 2.2%). In the rat, the ratios of S-100- and GFAP-positive cells were almost similar in NA-cell regions (S-100: 81.5 ± 2.6%; GFAP: 78.2 ± 2.3%), but difference in A-cell regions (32.4 ± 3.7%; 16.2 ± 2.5%, $P<0.0001$).

Conclusion: In both pigs and rats, the occurrence frequency of supporting cells was higher in NA-cell regions than in A-cell regions, and the numerical ratio of cells showing glial marker (S-100 protein), or GFAP in rats, to total nonchromaffin cells was higher in NA-cell regions than in A-cell regions. It seems likely that at least two subpopulations exist in supporting cells in rats, especially in A-cell regions.

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Key words: adrenal medulla, supporting cell, immunohistochemistry

Introduction

The adrenal medulla is the endocrine gland which originates from neuroectoderm, and contains at least two types of parenchymal cells, i.e., the chief amine-secreting type of cells (chromaffin cells) and other type of cells (supporting cells) (Coupland, 1984; Peters et al., 1991). Adrenomedullary chromaffin cells consist of adrenaline (A) cells and noradrenaline (NA) cells in many mammalian species. However, proportions and distribution patterns of these two types of chromaffin cells show marked species differences (Eränkö , 1955; Suzuki and Kachi, 1996). Supporting cells have features similar to those of glial cells in the nervous system (Coupland, 1984) and have been studied by electron microscopy and immunohistochemistry using the antibodies against glial marker proteins (Cocchia and Michetti, 1981; Coupland, 1984; Kachi et al., 1993; Suzuki and Kachi, 1994, 1995a, 1996). S-100 protein and glial fibrillary acidic protein (GFAP) used in this immunohistochemical study are now known as glial marker proteins, and are present in glia-like supporting cells in the rat and pig adrenal medullae.

We have been reported chiefly on the immunohistochemical qualitative findings of the relationship(s) between chromaffin cells and supporting cells in the adrenal medulla (Suzuki and Kachi, 1994, 1995a, 1996). In the present report, we will describe the quantitative differences in the cellular association(s) of chromaffin cells with supporting cells. A part of the abstract of preliminary results on this subject has appeared earlier (Suzuki and Kachi, 1995b).

1) Department of Physical Therapy, Faculty of Health Sciences, Aomori University of Health and Welfare, Mase 58-1, Hamadate, Aomori 030-8505, Japan

2) Department of Anatomy II, Hirosaki University School of Medicine, Zaifu-cho 5, Hirosaki, Aomori 036-8562, Japan

Materials and Methods

The present study was carried out on the adrenal medullae of 10-weeks-old male rat and 6-months-old male pig used in the previous study (Suzuki and Kachi, 1996). The process of preparing the sections and the immunostaining procedures have been detailed in our reports (Suzuki and Kachi, 1994, 1995a) and are reviewed here briefly. The sets of two adjacent sections were stained immunohistochemically using antisera to phenylethanolamine *N*-methyltransferase(PNMT) (Eugene Tech International, USA) and to one of two glial marker proteins, S-100 protein (DAKO Co., USA) or GFAP (Zymed Lab. Inc., USA), with the avidin-biotin peroxidase complex (ABC) method (Hsu et al. 1981). Primary antibodies were diluted at 1:1000 for PNMT and S-100 protein, 1:100 for GFAP, respectively.

One section was selected at random in S-100 protein- or GFAP-immunostained sections from the near-central portion in adrenal glands of 8 rats and 9 pigs. In each section, the numbers of chromaffin-cell (A- or NA-cell) nuclei and nonchromaffin-cell (S-100-protein- or GFAP-positive- and negative-cell) nuclei were counted in 5 or 6 areas in both A-cell and NA-cell regions with a light microscope. Over than 300 A-cell or NA-cell nuclei were counted in each area. The relative numbers or numerical ratios of several indexes as described in results were calculated. A and NA cells were determined by comparing with the adjacent section immunostained with anti-PNMT. Student-Fischer t-test was used for statistical analysis and for significance levels.

Results

Qualitative observations

The immunohistological findings as to PNMT-, S-100 protein-, and GFAP-immunoreactivity in the adrenal medullae of several mammalian species were previously reported (Suzuki and Kachi, 1994, 1995a, 1996). The characteristic features of qualitative results will now be described briefly. In the rat adrenal medulla, A cells (PNMT-positive cells) occupied large areas, and small or large islets of NA cells (PNMT-negative cells) were scattered among A-cell regions (Fig. 1, Type-I). In the pig, peripheral regions of the adrenal medulla were occupied with A cells, and central regions with NA cells(Fig.1,Type-II).PNMT-immunoreactivity was not found in adrenocortical cells. In NA-cell regions, S-100- or GFAP-positive cells were located in both inner and marginal portions of the parenchyma, and sent out fine cytoplasmic processes and formed a network by immunoreactive elements among NA-cells (Figs. 2a, 3a, 4).

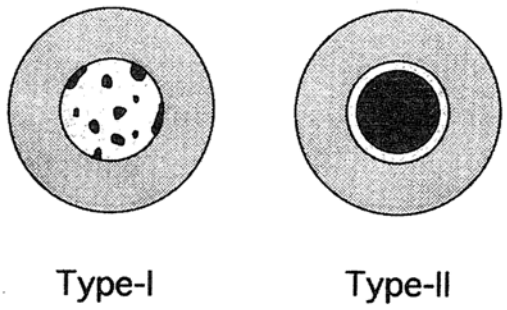


Fig. 1. Distribution patterns of A and NA cells in the rat (Type-I) and pig (Type-II) adrenal medullae . Dotted (gray) area shows adrenal cortex, and white and black areas show A-cell and NA-cell regions in the adrenal medulla, respectively.

In A-cell regions, S-100- or GFAP-positive cells were present very sparsely and not formed a network (Figs. 2b, 3b).

Quantitative observations

Relative proportion of NA-cell regions to total parenchymal-cell regions

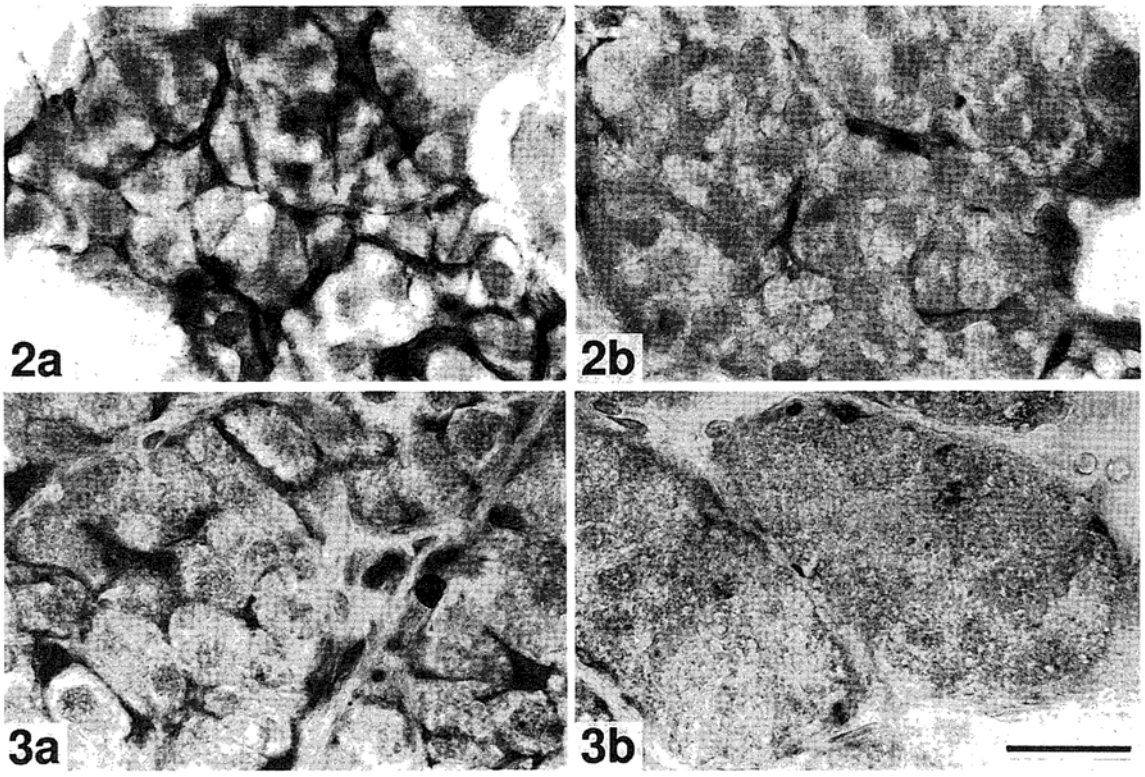
The pig adrenal medulla contains NA cells in much higher ratio than dose the rat adrenal medulla. The relative proportion of area of NA-cell regions to total area of both A- and NA-cell regions was 56.3 ± 2.3% (mean ± standard error) in the pig and 24.7 ± 3.2% in the rat ($P<0.001$), respectively.

Relative numbers of nonchromaffin cells to total intraparenchymal cells (Table 1)

The relative number of nonchromaffin cells to total intraparenchymal cells (nonchromaffin and chromaffin cells) in NA-cell regions was 20.2 ± 0.7% in the rat and 22.3 ± 1.3% in the pig, and that in A-cell regions was 7.9 ± 0.5% and 9.5 ± 0.7%, respectively. Therefore in both species, this index was higher in NA-cell regions than in A-cell regions (pig, rat: $P<0.0001$).

Numerical ratios of immunoreactive cells to total nonchromaffin cells (Table 2)

In the rat adrenal medulla, the numerical ratios of S-100 protein- and GFAP-immunopositive supporting cells to total nonchromaffin cells were almost similar in NA-cell regions (S-100 protein: 84.5 ± 2.0%; GFAP: 80.6 ± 1.7%), but in A-cell regions the ratio of S-100-positive cells was higher than twice as much as that of GFAP-positive cells (S-100 protein: 32.8 ± 3.3%; GFAP: 14.5 ± 2.3%, $P<0.0005$). In the pig, the ratio of S-100 protein-positive cells to total nonchromaffin cells was higher in NA-cell regions (91.0 ± 1.9%) than in A-cell regions (29.9 ± 2.2%).



Figs. 2a-3b.S-100 protein immunoreactivity in NA- (a) and A-cell (b) regions in the adrenal medullae of the rat (2) and pig (3). In NA- cell regions, S-100 protein immunoreactive supporting cells and their cytoplasmic processes are present more frequently than in A-cell regions. For GFAP immunoreactivity in both NA- and A-cell regions, see our previous report (Suzuki and Kachi, 1995a). Scale bar, 25 É m in Figures 2a-3b.

Fig.4. LM tracing of NA-cell islands. Nuclei, immunoreactive cytoplasm, and cytoplasmic processes of S-100 protein immunoreactive supporting cells are shown by dotted area, black area, and line, respectively. NA-cell nuclei and the margin of the parenchyma are shown by circle and short dashed line, respectively. A-type: tracing of the pig adrenal medulla. B-type: tracing of the rat adrenal medulla. For more details, see text.

Numerical ratios of S-100-positive cells located in inner and marginal portions of the parenchyma (Table 3)

In NA-cell regions, S-100-positive cells were relatively densely located in both inner (55.8 ± 1.2%) and marginal (44.2 ± 1.2%) portions of the parenchyma in the pig adrenal medulla, but in the rat these were located more frequently in the inner (72.0 ± 1.5%) than in the marginal (28.0 ± 1.5%) portions. In A-cell regions, the numerical ratios of these cells located in the inner and marginal portions were 28.2 ± 3.1 % and 71.8 ± 3.1% in the pig, and 56.0 ± 5.2 % and 44.0 ± 5.2% in the rat, respectively.

Table 1. Relative and actual (in parentheses) numbers of nonchromaffin cells to total intraparenchymal cells.

Species	NA-cell Regions	A-cell Regions
Rat	20.2±0.7[a]	7.9±0.5[a]
(n=8)	(88/435)	(38/481)
Pig	22.3±1.3[b]	9.5±0.7[b]
(n=9)	(102/457)	(29/305)

n:number of animals, Mean ± standard error, (/):mean actual number
Student-Fischer t-test : a, b, P<0.0001

Table 2. Relative and actual (in parentheses) numbers of immunoreactive cells to total nonchromaffin cells.

	NA-cell regions		A-cell regions	
Species	S-100	GFAP	S-100	GFAP
Rat	84.5±2.0	80.6±1.7	32.8±3.1[a]	14.5±2.3[a]
(n=8)	(75/88)	(71/88)	(12.5/38)	(5.5/38)
Pig	91.0±1.9	—	29.9±2.2	—
(n=9)	(93/102)		(8.5/29)	

n : number of animals, Mean ± standard error, (/) : mean actual number
Student-Fischer t-test : a, P<0.0005

Table 3. Relative and actual (in parentheses) numbers of S-100-positive cells located in inner (Ip) and marginal (Mp) portions of the parenchyma.

	NA-cell regions		A-cell regions	
Species	Ip	Mp	Ip	Mp
Rat	72.0±1.5	28.0±1.5[a]	56.0±5.2	44.0±5.2
(n=8)	(54/75)	(21/75)	(7/12.5)	(5.5/12.5)
Pig	55.8±1.2	44.2±1.2[a]	28.2±3.1	71.8±3.1
(n=9)	(52/93)	(41/93)	(2.4/8.5)	(6.1/8.5)

n : number of animals, Mean ± standard error, (/) : mean actual number
Student-Fischer t-test : a, P<0.0001

Discussion

S-100 protein was first described by Moore (1965) as the cytoplasmic calcium-binding protein extracted from bovine brain, and Cocchia and Michetti (1981) found the presence of this protein immunohistochemically in the supporting cells in the rat adrenal medulla. GFAP is one of the intermediate filament proteins which are included in the glial filaments, and it has recently been shown that GFAP and/or GFAP-like peptides are present not only in the central nervous system but also in the peripheral nervous system (Jessen and Mirsky, 1980, 1983; Kobayashi et al., 1986; Mokuno et al., 1989). Now, these two proteins are known as two different types of glial marker proteins.

In our previous qualitative study, the occurrence of S-100 protein immunoreactive cells were apparently more frequent in NA- than in A-cell regions in seven mammalian species, irrespective of species differences in relative concentration of NA (Holzbauer and Sharman, 1972), volumetric proportions

and/or distribution patterns of two types of chromaffin cells (Eränkö , 1955; Suzuki and Kachi, 1996). In this quantitative study, relative numbers of nonchromaffin cells to total intraparenchymal cells and numerical ratios of glial marker immunopositive cells to total nonchromaffin cells were higher in NA-cell regions than in A-cell regions. The present results support the previous qualitative results in both pig and rat, and it may be expected to obtain similar numerical ratios in other mammalian adrenal medullae.

On the other hand, in the rat adrenal medulla the ratios of S-100 protein- and GFAP-immunoreactive cells to total of nonchromaffin cells were almost similar in NA-cell regions, but in A-cell regions the ratio of S-100 cells was almost twice as much as that of GFAP cells. From these findings, it seems likely that at least two subpopulation types of glia-like supporting cells, showing [S-100-positive and GFAP-positive] and [S-100-positive but GFAP-negative], exist especially in A-cell regions in the rat adrenal medulla. The positional difference(s) in chemical nature between supporting cells located in inner and marginal portions of the parenchyma was not shown in A- and NA-cell regions in both species.

The cell membranes of A cells and NA cells, having characteristics of endocrine cells, expose to the pericapillary space directly. However, supporting cells surround large surface areas of NA cells than of A cells. While, the numerical ratio of S-100-positive cells located in marginal portion of the parenchyma in NA-cell regions is higher in the pig than in the rat adrenal medulla. This may mean that in the pig the external surfaces of NA-cell islands are largely covered by supporting cells and the cell membranes of NA cells are separated from the pericapillary space by lamellar processes of those supporting cells than in the rat.

The degree of cellular association between adrenomedullary chromaffin cells and supporting cells was denser and closer in NA-cell regions than in A-cell regions. And the species difference was present in the location of supporting cells in the parenchyma in NA-cell regions between the pig and rat adrenal medullae. The former morphological features were commonly found in the adrenal medullae having both A cells and NA cells irrespective of, but the latter may be caused by, species differences in proportions and distribution patterns of A and NA cells. However, such arrangements may as well represent a more detailed or graded control of NA release as compared with A release in the adrenal medulla, and also compared with NA release in the rat. The biological and functional significances of these structural differences have been discussed in our previous reports (Suzuki and Kachi, 1994, 1995a, 1996), but the role of supporting cells in the adrenal medulla and/or in differences in functions between A and NA cells still remain to be established.

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