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〔Original Paper〕

Immunohistochemical studies on supporting and chromaffin cells in the Chinese hamster adrenal medulla in the prehibernating season

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Abstract

The adrenal medulla of the Chinese hamster, one of the hibernating rodents, was used. Cryostat sections fixed with 4% paraformaldehyde or Zamboni fluid were immuno stained by the labelled streptavidin biotin (LSAB) method using antisera to S-100 protein and phenylethanolamine N-methyltransferase (PNMT). PNMT-immunoreactive cells (adrenaline (A) cells) were observed mainly in central regions, and PNMT-immunonegative cells (noradrenaline (NA) cells) chiefly in peripheral regions of the adrenal medulla. S-100 protein-immunoreactive cells were more numerously and densely distributed in NA- than in A-cell regions. In NA-cell regions, S-100 protein-labelled cells were present almost evenly in both marginal and inner portions of the parenchyma, and immunoreactive elements of supporting cells formed the coarse network. In A-cell regions, S-100-positive cells were scarcely found. This close cellular association of supporting cells with NA cells in the adrenal medulla may reflect the biological and functional characteristics in the prehibernating season.

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Keywords: adrenal medulla, supporting cell, Chinese hamster

Introduction

The adrenal medulla is one of the amine-secreting endocrine glands, having a neuroectodermal origin. Its hormone, adrenaline, is finally synthesized by phenyl ethanolamine N-methyltransferase (PNMT). In the adrenal medulla, there are at least two types of parenchymal cells, i.e., the chief amine-secreting type of cells (chromaffin cells) and another type of cells (supporting cells) (Coupland, 1984; Peters et al., 1991). The chromaffin cells consist of adrenaline (A) cells, which have PNMT activity, and noradrenaline (NA) cells, which have no PNMT activity, in many mammalian species.

The Chinese hamster adrenal medulla has been very little studied. In the present study, we examined it immunohistochemically in two ways: first, the distribution pattern of two types of chromaffin cells; and second, the extent of cellular associations of supporting cells with A and NA cells in the adrenal medulla of the prehibernating Chinese hamster.

Materials and Methods

All animal experiments in this study followed the "Guidelines for animal experimentation" as laid down by the Animal Research Committee, Aomori University of Health and Welfare.

Three adult male Chinese hamsters in the autumn (before hibernation), inhabiting in the Province of Jilin, China, were used in this study. The animals were killed by decapitation with minimal disturbance. The adrenal glands were removed immediately and cut into halves. One or two adrenal blocks of each animal were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) and the other blocks with Zamboni fluid at room temperature. After rinsing overnight in PB containing 5% (w/v) sucrose, the adrenal blocks were immersed in 30% sucrose in PB for 24 h at 4℃. The blocks were embedded in O.C.T. compound (Miles Scientific Co.) and frozen rapidly in liquid nitrogen. The frozen blocks were cut into serial sections of 10μm in thickness with a Cryostat and mounted on poly-L-lysine-coated glass slides.

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Immunohistochemistry

Sections were stained immunohistochemically using antisera to phenylethanolamine N-methyltransferase (PNMT) (Eugene Tech International, USA) or to one of the glial marker proteins, S-100 protein (DAKO Co., USA), with the labelled streptavidin biotin (LSAB) method using a commercially available LSAB kit (DAKO Co., USA). Primary antibodies were diluted at 1:1000 with 0.01M PB saline (pH.7.4) containing 0.5% Triton X-100 and 0.1% bovine serum albumin. The process of immunostaining has been detailed in our previous report (Suzuki and Kachi, 1994, 1996).

Results

The following findings were obtained by careful examination of sets of two adjacent sections, the one immunostained for PNMT and the other for S-100 protein.

Anti-PNMT (Fig. 1). Adrenomedullary A cells are positively immunostained with anti-PNMT, but NA cells are negative for PNMT. In the Chinese hamster adrenal medulla, PNMT-immunoreactivity could be observed mainly in central chromaffin cells and less frequently in peripheral ones adjacent to cortical cells. Most of the chromaffin cells in the peripheral regions of the adrenal medulla were not immunostained with anti-PNMT. Small or large islets of immunonegative cells were scattered among central medullary cells. Therefore, in the adrenal medulla of the Chinese hamster, the central regions correspond chiefly to A-cell regions, and the peripheral regions to NA-cell regions. PNMT-immunoreactivity was not found in any of the adrenocortical cells.

S-100 protein (Figs. 2a, b). The immunohistochemical reactions to anti-S100 protein were negative in adrenocortical cells and adrenomedullary chromaffin cells but were intensely positive in stellate shaped cells scattered among chromaffin cells in the adrenal medulla. The occurrence of S-100 protein immunoreactive (supporting) cells was apparently more frequent in PNMT-negative (NA-cell) regions than in PNMT-positive (A-cell) regions. In NA-cell regions, S-100 labelled cells were located in both inner and marginal portions of the parenchyma, extending fine cytoplasmic processes between NA cells and forming a coarse network by immunoreactive elements among NA-cells (Fig. 2a). In A-cell regions, S-100-positive cells were present very sparsely and did not form a network (Fig. 2b).

Discussion

The proportions and distribution patterns of A cells and NA cells in the adrenal medulla have been studied in many mammalian species, using histochemical or immunohistochemical techniques. (Eränkö, 1955; Hillarp and Hökfelt, 1955; Wood, 1963; Coupland, 1989; Suzuki and Kachi, 1996). In previous reports, we summarized the distribution patterns of these two types of chromaffin cells and classified them into five cardinal types (Suzuki and Kachi, 1996; Kachi et al., 1998). In the rat, mouse, cat, dog, and some others mammalian adrenal medullae, A cells occupy large areas and small or large islets of NA cells are scattered among A-cell regions. In the golden hamster, NA cells exclusively distribute in the juxtacortical region. The distribution pattern of the Chinese hamster used in this study seems to be classified into the intermediate or transitional type between the above two types, because NA cells were observed chiefly in peripheral regions and their small and large islets in central A-cell regions.

In the Chinese hamster adrenal medulla, the occurrence of S-100 protein immunoreactive (supporting) cells was apparently more frequent in the NA-cell region than in the A-cell region, similar to the results in seven mammalian species previously examined (Suzuki and Kachi, 1996). These characteristics show that the degree of cellular association between supporting cells and chromaffin cells is higher in NA- than in A-cell regions, irrespective of species differences in relative concentration of NA (Holzbauer and Sharman, 1972), in volumetric proportions and/or distribution patterns of two types of chromaffin cells. Such arrangements may as well represent a more detailed or graded control of NA release as compared with A release in the adrenal medulla.

During the transition to and the period of hibernation, the epinephrine levels in both plasma and adrenal gland decrease in the garden dormouse, being one of the hibernating rodents (Atgie et al., 1990). For these reasons, Atgie et al. evaluate the link between the spontaneous changes in food intake and the activity of the peripheral sympathetic nervous system. Moreover, Avakian and Horvath (1981) reported that norepinephrine turnover in rats fell with fasting and that a fall in plasma glucose levels is associated with sympathetic nervous system activity reduction. It is very interesting to examine and elucidate morphologically whether seasonal differences are present in the distribution patterns of A and NA cells and/or in the interrelationships between supporting cells and chromaffin cells in both A- and NA-cell regions of the Chinese hamster adrenal medulla.

The biological and functional significances of these structural differences have been discussed in our previous reports, but the role of supporting cells in the adrenal medulla and/or

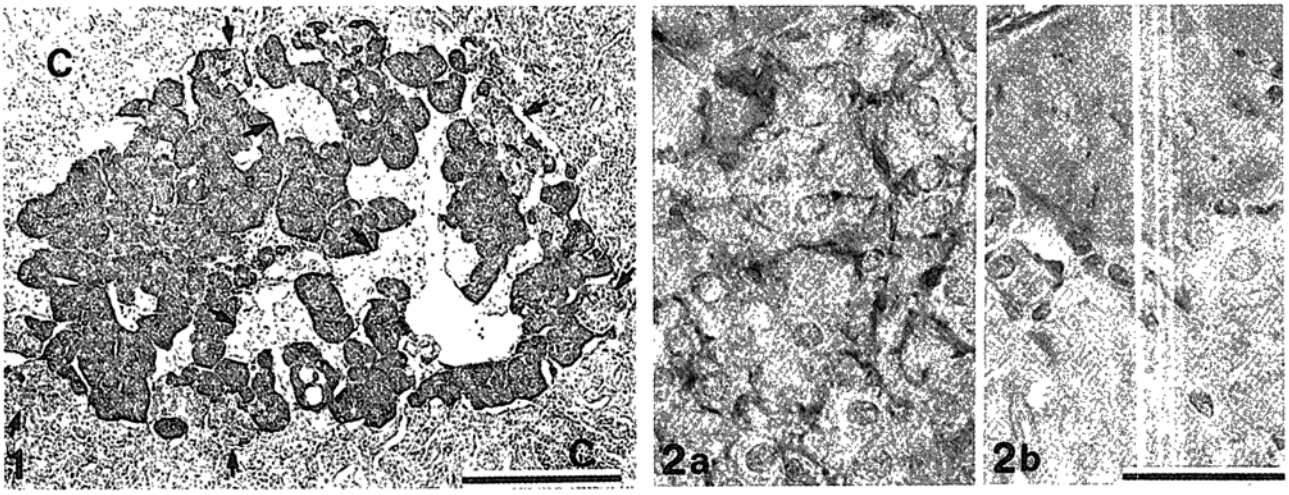

Fig. 1. PNMT-immunoreactivity in the Chinese hamster adrenal medulla. A cells immunostained with anti-PNMT. Adrenocortical cells (C) and NA cells (arrow) were PNMT-negative. Bar=300μ m.

Figs. 2a, b. S-100 protein immunoreactivity in the Chinese hamster adrenal medulla. In NA-cell regions (a), S-100 protein immunoreactive supporting cells and their cytoplasmic processes are present more frequently than in A-cell regions (b). Bar=45μ m.

in differences in functions between A and NA cells still remain to be established.

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