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Quantitative electron-microscopic study on glial cells in contact with the perivascular space in the rat pineal gland: Effects of intracranial surgery

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Abstract

Glial cells in the rat pineal gland of normal and sham-pinealectomized (SPX) groups were examined electron microscopically, 14 days after surgery. The relative lengths of glial cell membrane profiles adjoining the basal lamina of the perivascular spaces were significantly decreased by surgery. The relative numbers of glial cells showed similar tendency. It is suggested that such anatomical rearrangement may facilitate the release of pineal homone(s) into, and influences of regulatory and/or modulatory substances from, the perivascular spaces.

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Key words: pineal gland , glial cell , perivascular space

Introduction

The pineal gland is an amine-secreting endocrine gland, having a neuro-ectodermal origin embryologically, and contains at least two types of parenchymal cells: pinealocytes and glial cells [7, 12]. In the central nervous system and neuroendocrine system, the close cellular association of neurons and glia is a familiar notion, and neuron-glia interactions and their structural plasticity are considered to be important in regulating activities of both neurons and glia [10]. From these aspects, it would be possible that similar interrelationships between pinealocytes and glial cells are present.

Many papers on pinealocytes have appeared [3, 12], but there has been only one, as far as we know, electron microscopic study as to the experimental influence on the glial cells in the pineal gland [1]. On the other hand, our data concerning pineal effects on morphological components of the adrenal medulla indicate that the direction of sham pinealectomy (SPX) effects is usually opposite to that of pinealectomy (PX) effects [2]. Therefore, we sought to clarify electron microscopically whether and how glial cells in the pineal gland are influenced by intracranial surgery. In the present study, considering the report on changes in the perivascular contact area after adrenalectomy [1], we measured the length and number of glial elements contacting the perivascular space and compared those between the normal and SPX groups of rats. A part of the present results has appeared earlier [9].

Materials and Methods

Six male Wistar rats obtained at 4 weeks of age were maintained with free access to food and water in a windowless animal room with controlled temperature (22±2℃) and light (LD 12:12). Three animals were sham-pinealectomized (SPX) at 5 weeks of age in accord with procedures described previously [8]. After an additional 14 days, three normal (intact, non-operated) and three SPX animals were sacrificed at 4 hr after the onset of darkness by decapitation. The pineal gland was removed immediately and fixed with 2.5% glutaraldehyde in 0.05M phosphate buffer for 1 hr, and postfixed with 1% OsO_4 for 1.5 hr, then dehydrated in an ethanol series and embedded in Epon. Sections, about 70 nm

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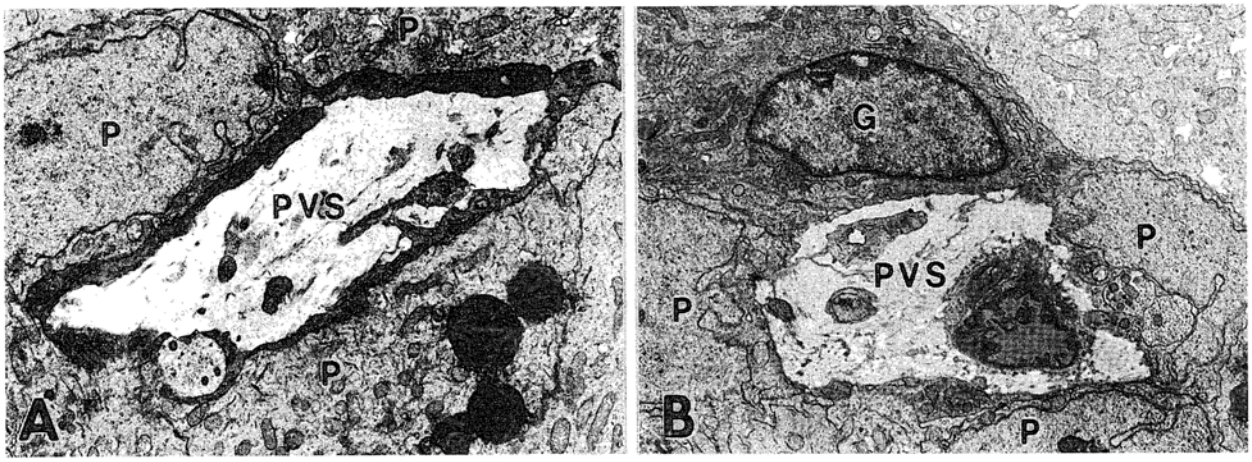

Fig. 1 Perivascular space (PVS) and their surrounding structures in the distal region of the rat pineal gland. P, pinealocyte; G, glial cell. X4,400
A: Normal rat. Pinealocytes are separated form the perivascular space by a barrier of electron-dense glial elements and pinealocyte-perivascular contact area is narrow.
B: SPX rat. Compared with normal rats, glio-perivascular contact area diminishes and the degree of exposure of pinealocytes to the perivascular space increases.

in thickness, were mounted on 200-mesh grids. Following double staining with uranyl acetate and lead citrate, sections were examined under the electron microscope.

38 perivascular spaces and their surroundings of normal animals and 33 of SPX animals were photographed at a magnification of about X3,000. Enlarged photomontages in a total magnification of X7,800 were used for detailed observation. The lengths of cell membrane profiles of glial cells (and/or terminals of glial cell processes) and pinealocytes (and/or pinealocyte processes) adjoining the basal lamina of the perivascular space were measured using a cartographer's curve-meter and by an image analyzer (IBAS, Carl Zeizz), and the number of glial cells and pinealocytes were counted and calculated as the percentage of these two types of cells relating to the perivascular space. In this study, these measurements were made mainly in the distal region of the body portion of the pineal gland. Student-Fischer *t*-test was used for statistical analysis and for significance levels.

Results

Qualitative observations

Glial cells and their cytoplasmic laminar processes showed markedly electron-dense appearance partly due to their richness in microfilaments and were observed near to, or in contact with, the perivascular space. In comparison to pinealocytes, glial cells were slender and had a rather dark nucleus, because of their large amount of condensed chromatin, and a smaller nucleolus. Glial-cell nuclei were oval and had a thin but conspicuous rim of heterochromatin inside the nuclear membrane, but the pinealocyte apparently lacked this feature (Fig. 1). In the normal rat, the pinealocytes were separated from the perivascular space by the barrier of glial cells and/or their cytoplasmic processes and the pinealocyte-perivascular contact area was narrow (Fig. 1A). After the SPX treatment, glio-perivascular contact area diminished and the degree of exposure of pinealocytes to the perivascular space increased (Fig. 1B).

Quantitative observations

In the normal rat, the relative length of glial cell membrane profile to total length of pinealocyte and glial cell membrane profiles facing the basal lamina of the perivascular space was $58.5 \pm 3.1\%$ (mean $\pm$ standard error), in the SPX rat this value significantly decreased to $43.6 \pm 3.0\%$ ($P<0.002$). This is to say, the relative extent of pinealocyte and their processes exposed to the perivascular space increased by almost 15% in the SPX rat (Table 1). The relative numbers of glial cells (and/or terminals of glial cell processes) and pinealocytes (and/or pinealocyte processes) abutting to the perivascular space were $47.1 \pm 1.9\%$ and $52.9 \pm 1.9\%$ in the normal rat, and $39.5 \pm 2.0\%$ and $60.5 \pm 2.0\%$ in the SPX rat, respectively (Table 1). In the normal rat, there was no difference between the relative numbers of two types of cells surrounding the perivascular space, but in the SPX rat this difference was statistically significant at $P<0.001$ level. The difference in the relative number of glial cells between the normal and SPX rats was significant ($P<0.01$).

Discussion

Our present results clearly showed that the glial coverage of perivascular spaces in the pineal gland diminishes the area

Table 1. Relative lengths and numbers of glial cells and pinealocytes (in parentheses) in contact with the perivascular space.

	Length	Number
Control (38)	58.5±3.1[a]	47.1±1.9[b]
	(41.5±3.1)	(52.9±1.9)
SPX (33)	43.6±3.0[a]	39.5±2.0[b,c]
	(56.4±3.0)	(60.5±2.0)[c]

Student-Fischer t-test: a, P<0.002; b, P<0.01; c, P<0.001
( ): number of capillaries, Mean ± standard error

following the SPX treatment. According to Deuβen-Schmitter et al. [1], the perivascular region shows a significant change in the ratio of glial elements contacting with the perivascular space in bilateral adrenalectomized rat pineal glands. While 40% of the contact area is occupied by pinealocytes and 60% by glial cell processes in normal rats, this proportion is reversed 14 days after bilateral adrenalectomy. Thus, bilateral adrenalectomy as well as SPX induces a decrease in the glio-vascular contact area in the pineal gland.

It has been shown that physiological and/or physical stimuli induce neurone-glia morphological changes in several neuroendocrine systems other than pineal gland, and lead to an increased neuro-vascular contact area [5, 10, 11, 13, 14]. These and our present results indicate the following possibility that the degree of exposure of pinealocytes to the perivascular space increases under the states of increased functional activities of the pineal gland. Supporting evidence has been reported in sea turtles that the plasma melatonin levels increase in SPX animals [6]. However, clear-cut data have not been obtained in rats, although similar trends have been observed [4].

As the possible functional significance of glial plasticity, it is surmised that the enlarged exposure of pinealocytes to the perivascular space in SPX animals would facilitate for pinealocytes not only to release hormonal substance(s) into, but also to receive influences of neural, hormonal and other local factors from, the perivascular space. Although it is postulated that the retraction of glial cell processes and enlargement of pinealocytes and/or their cytoplasmic processes in SPX animals, much remains to be done also about details of morphological plasticity in the surroundings of pineal capillaries following the SPX treatment, such as three-dimensional changes in the shape and intracellular structure of each glial cell or pinealocyte.

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ラット松果体におけるグリア性支持細胞の電顕的研究

― 頭蓋内手術の影響 ―

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神経組織に由来する松果体において、グリア性支持細胞に松果体除去頭蓋内対照手術（SPX）の影響が見られるか否かを電顕的に検索・比較した。24時間明暗周期、22±２℃の恒温環境で飼育し、５週齢でSPXを行い、術後２週間の暗期開始後４時間で屠殺した正常（NO）群、SPX群の雄性ラット各３匹を用いた。２.５％GAと１％OsO４で固定した後、通常の方法で松果体電顕モンタージュ写真（倍率7,800倍）を作製、血管周囲腔を被覆するグリア性細胞と松果体細胞それぞれの膜の長さと細胞断面数を計測した。血管周囲腔を被覆するグリア性細胞の周長に対する比率は、SPX群（N、X±SE：33、43.６±３.０％）でNO群（38、58.５±３.１％）よりも有意の低値を示した（t検定：P<０.001）。グリア性細胞と松果体細胞断面の数的比率には、NO群（47.１±１.９％、52.９±１.９％）で差異は見られなかったが、SPX群（39.５±２.０％、60.５±２.０％）で有意差が見られ（P<０.001）、両細胞の実験群間差も有意であった（P<０.01）。実験成績より、SPXは血管周囲腔を被覆するグリア性細胞の形状や配置に影響し、松果体細胞からの分泌物の血管周囲腔への放出を容易にする可能性が示唆されたが、今後更に微細構造も含めて検討する必要があると考えられた。

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キーワード：松果体，グリア性細胞，血管周囲腔