Effect of superior cervical ganglionectomy on the ultrastructure of pinealocytes in the Djungarian hamster (*Phodopus sungorus*): Quantitative study

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Abstract

OBJECTIVES: Superior cervical ganglia are of crucial importance in regulating the secretion of the pineal hormone – melatonin. Changes in pineal morphology have been observed in many mammalian species after sympathectomy. Therefore, we decided to investigate the ultrastructure of Djungarian hamster pinealocytes following superior cervical ganglionectomy.

MATERIAL AND METHODS: Eight adult female Djungarian hamsters (*Phodopus sungorus*) were used in this study. The superior cervical ganglia were removed bilaterally in four animals, whereas the other four animals served as sham-operated controls. The pineal glands were removed four weeks after the operation and processed for electron microscopic study. The cross-sectional areas of pinealocyte and its nucleus, and relative volume of mitochondria, Golgi apparatus, lysosomes, granular endoplasmic reticulum, and glycogen particles as well as the numbers of dense-core vesicles and "synaptic" ribbons were estimated using a digital analyzer connected on-line to IBM-PC computer. Statistical analysis of the data was performed using Student's t test and Snedecor F test.

RESULTS: Significant reduction in the cross-sectional areas of the pinealocyte and its nucleus as well as in the relative volumes of mitochondria and glycogen particles was observed after superior cervical ganglionectomy in comparison with sham-operated controls. Sympathectomy resulted also in reduction of the number of dense-core vesicles. On the contrary 2,5 fold increase in the number of "synaptic" ribbons was observed in ganglionectomized animals in comparison to sham-operated controls.

CONCLUSION: Deprivation of sympathetic innervation leads in Djungarian hamsters not only to suppression of melatonin synthesis and secretions but, as appears from our studies, induces also morphological changes suggesting lower metabolic and secretory activity of pinealocytes.

ORIGINAL ARTICLE

Introduction

The mammalian pineal gland is innervated primarily, if not entirely, by postganglionic sympathetic nerve fibers arising from neurons located in the superior cervical ganglia [1, 2]. These nerve fibers play a crucial role in regulation of pineal function. They contain and release norepinephrine which stimulates the synthesis of the pineal hormone – melatonin [3, 4]. However, ultrastructural studies, especially those using quantitative methods, on the effects of sympathectomy on the pineal cells are rare. Therefore, the aim of the present study was to investigate the ultrastructure of pinealocytes in the Djungarian hamster following bilateral superior cervical ganglionectomy.

Material and methods

Eight adult female Djungarian hamsters (*Phodopus sungorus*) were used in this study. The animals were housed under controlled illumination (L:D 14:10) and temperature (22±2ºC).The superior cervical ganglia were removed bilaterally in four animals, whereas the other four animals served as sham-operated control. Four weeks after the operation the animals were anaesthetized with ether between 10:00 h and 11:00 h. They were fixed by cardiac perfusion with 3.5% glutaraldehyde:2% paraformaldehyde in 0.067 M cacodylate buffer for 10–15 min. The pineal glands were subsequently removed and fixed for an additional 2 h, postfixed in 1% osmium tetroxide and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate and examined with JEOL 100B electron microscope.

For quantitative estimation 5 to 7 micrographs at magnification of x3,000, and 30 to 25 micrographs at magnification of x 10,000 were taken from each gland using slightly modified systematic random sampling method of Weibel. A digital analyzer connected on-line to IBM-PC computer (Logitex, Poland) was used to obtain the morphometric data. The cross-sectional areas of pinealocyte and its nucleus, and relative volume of mitochondria, Golgi apparatus, lysosomes, granular endoplasmic reticulum, and glycogen granules as well as the number of dense-core vesicles were estimated on the prints enlarged photographically by 2.5 x. For quantification of "synaptic" ribbons the tissue overlying 10 grid apertures, each measuring 45 x 45 ìm^2, was scanned at x 15,000.

Statistical analysis of the data was performed using Student's t test and Snedecor F test.

Results

Significant reduction in the cross-sectional areas of the pinealocyte and its nucleus (Fig. 1) as well as in the relative volumes of mitochondria and glycogen particles (Fig. 2) was observed after superior cervical ganglionectomy in comparison with sham-operated controls. Sympathectomy resulted also in reduction of the number of dense-core vesicles (Fig. 3). On the contrary 2,5 fold increase in the number of "synaptic" ribbons was observed in ganglionectomized animals in comparison with sham-operated controls (Fig. 3). No differences were noted in the relative volumes of Golgi apparatus, granular endoplasmic reticulum, and lysosomes (Fig. 2).

Discussion

The results of the present study indicate that loss of sympathetic innervation resulted in morphological features which, in functional sense, can be interpreted as a reduction of metabolic and secretory activity of pinealocytes in the Djungarian hamster. This is exemplified by reduction in the size of pinealocytes and their nuclei as well as in the relative volumes of mitochondria and glycogen particles, and decrease in number of dense-core vesicles. This finding corroborate with morphological patterns of diminished metabolic and secretory activity of pinealocytes after sympathectomy demonstrated qualitatively in the rabbit [5], and golden hamster [6], and quantitatively in the gerbil [7], cotton rat [8], and rat [9].

Quantitative changes in mitochondria which in the pinealocyte, as in all eukariotic cells, play a well-known role in cellular metabolism (i.e. conservation of oxida-

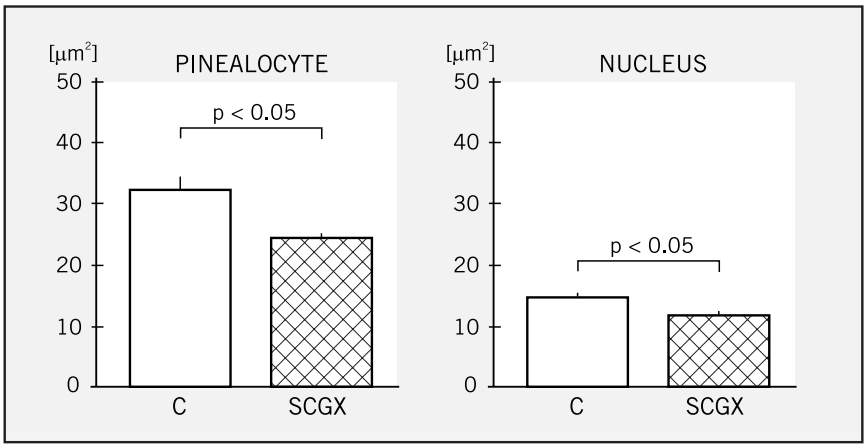

Fig. 1. Cross-sectional areas of the pinealocyte and its nucleus in sham-operated (C) and ganglionectomized (SCGX) Djungarian hamsters.

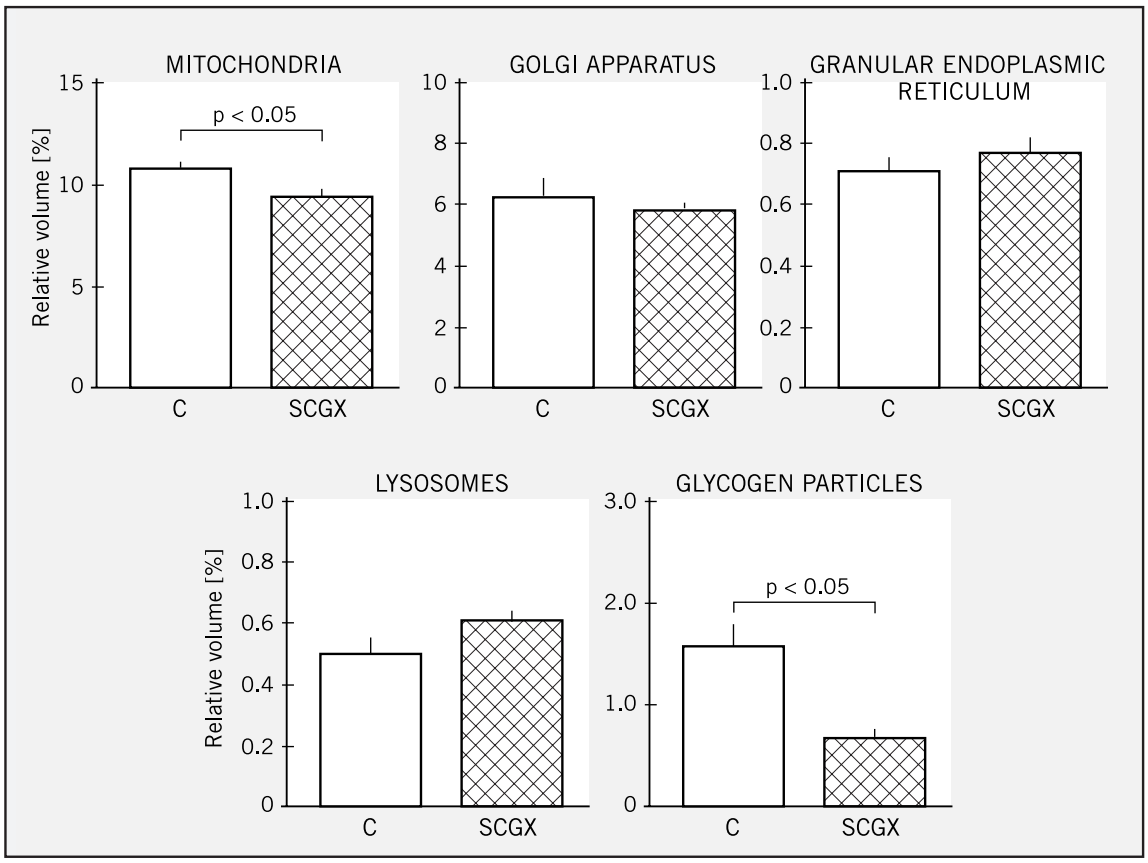

Fig. 2. The relative volumes of mitochondria, Golgi apparatus, granular endoplasmic reticulum, lysosomes, and glycogen particles in pinealocytes of sham-operated (C) and ganglionectomized (SCGX) Djungarian hamsters.

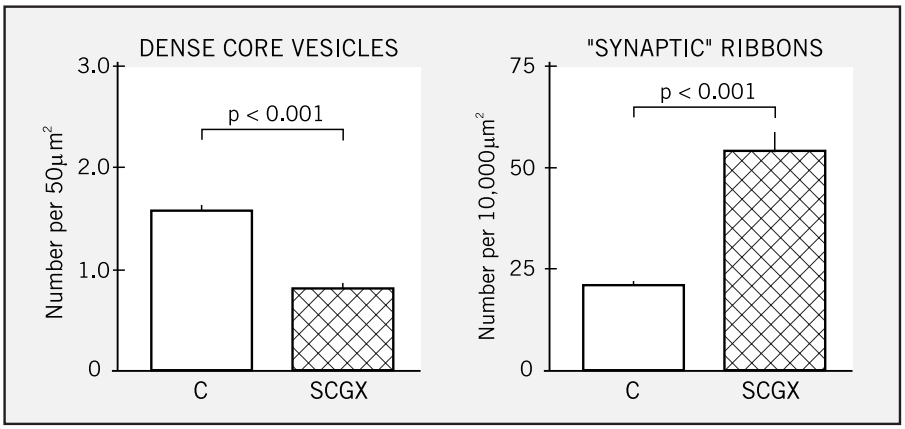

Fig. 3. The number of dense-core vesicles and "synaptic" ribbons in pinealocytes of sham-operated (C) and ganglionectomized (SCGX) Djungarian hamsters.

tively derived energy and its utilization for ATP synthesis) are good indicators of cell activity. Although the presence of glycogen particles, which are abundant in the Djungarian hamster pinealocytes, is not a regular feature of these cells in various species, and its relationship to pineal biochemistry is not yet known, quantitative changes in glycogen deposits may also reflect biochemical status of the cell.

Although the exact nature of the dense-core vesicles content is unknown these structures, showing distinct numerical changes in many natural and experimental conditions [10], are by many authors linked to pinealocyte secretory activity [2, 11–14]. Like in the Djungarian hamster decrease in the number of dense-core vesicles after sympathectomy has been observed in majority of examined species, including the rabbit [5], golden hamster [6], mouse [15], and cotton rat [8]. In contrast, an increase in the number of dense-core vesicles has been demonstrated after electrical stimulation of the superior cervical ganglion in the rat pinealocytes [16] and following addition of norepinephrine to the cultured pinealocytes of the rat [17] and rabbit [18].

"Synaptic" ribbons in the mammalian pinealocytes are apparently related to the pineal adrenergic innervation [2]. The present study confirmed our earlier observation that the number of "synaptic" ribbons markedly

increase following sympathectomy [19]. An inverse correlation between the density of adrenergic nerve fibers and "synaptic" ribbons number has been demonstrated in the pineal gland of diverse number of mammalian species, including the Djungarian hamster [20]. Elevated number of "synaptic" ribbons has been also resulted by sympathectomy in the pineal gland of the rabbit [5], rat [21], cotton rat [8], and cat [22] as well as by chemical sympathectomy in the rat [23]. Increased number of "synaptic" ribbons has been also found in the rabbit [24] and rat [25] pinealocytes in organ culture, i.e., in the pineal gland which are essentially denervated. Moreover, co-culture of the pineal gland with superior cervical ganglia or culture with addition of norepinephrine caused significant decline in "synaptic" ribbons number [26]. All these results indicate a compensatory increase in "synaptic" number in response to loss of adrenergic innervation.

In conclusion, deprivation of sympathetic innervation leads in Djungarian hamsters not only to suppression of melatonin synthesis and secretions but, as appears from our studies, induces also morphological changes suggesting lower metabolic and secretory activity of pinealocytes.

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