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Cervical sympathectomy inhibits axonal transport of gonadotropin-releasing hormone during continuous exposure to light in male rats

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Abstract: To examine the effects of cervical sympathectomy on the transport of gonadotropin-releasing hormone (GnRH) between the hypothalamic neurons and the median eminence, 16 male rats were assigned into four groups: control (C), light (L), light-sympathectomy (LS), and light-colchicine (LC). The C group was kept under a normal circadian rhythm for 2 weeks, and the L group was kept under continuous exposure to light for the same period. The LS group underwent bilateral cervical sympathectomy before being kept under continuous light conditions for 2 weeks. The LC group received colchicine into the cerebral ventricle after being kept under continuous light for 12 days; subsequently, this group was also housed for 2 days under continous light. After these procedures, blood was collected and serum luteinizing hormone (LH) levels were measured. All rats were perfused with a fixative, and GnRH neurons around the anterior commissure, as well as GnRH fibers and granules in the median eminence, were stained immunohistochemically. The L group showed a decreased number of GnRH neurons, increased concentrations of GnRH fibers and granules, and an increased LH level; however, in the LS and LC groups, these changes were not seen. The response in the LS group resembled that in the LC group. Considering the action of colchicine, which inhibits axonal transport, it is suggested that cervical sympathectomy also inhibits axonal transports of GnRH between the GnRH neurons and the median eminence during continuous exposure to light.

Key words: Cervical sympathectomy, Gonadotropin-releasing hormone, Hypothalamus, Median eminence, Rat

Introduction

In a previous report [1], we demonstrated that continuous exposure to light increases gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), and testosterone secretion in male rats. We also showed that bilateral cervical sympathectomy under continuous light conditions inhibits these hormonal changes, while it does not affect them under a normal circadian rhythm. GnRH neurons are located around the anterior commissure between the medial septal area and the medial preoptic area where GnRH is produced and transported to the median eminence and then secreted into the pituitary portal veins. In the present study, we examined the histological changes in GnRH neurons, and in fibers or granules in the hypothalamus and the median eminence, during cervical sympathectomy, and considered the mechanism of cervical sympathectomy in the male rat.

Materials and methods

Sixteen adult male Wistar rats weighing 250 ± 20g (mean ± SD) were used, and this study was conducted in accordance with the Fukushima Medical College. Guidelines on Animal Experiments, the Japanese Animal Protection and Management Law (No. 105), and the Japanese Regulations on the Feeding and Safekeeping of Animals (No. 6). All rats were given access to food and water ad libitum.

The rats were randomly assigned into four groups of four rats each: control (C), light (L), light-sympathectomy (LS), and light-colchicine (LC) groups. The C group was kept under a normal circadian rhythm for 2 weeks, and the L group was kept under continuous exposure to light for the same period. In the LS group, both inferior extremities of the superior cervical ganglia were cut off under pentobarbital 50 $mg \cdot kg^{-1}$ i.p. and atropine sulfate 0.05 $mg \cdot kg^{-1}$ i.m.; the group was then kept under continuous light conditions for 2 weeks. The LC group received colchicine 100μg into the cerebral ventricle under the same anesthesia after being

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kept under continuous light for 12 days; subsequently, this group was also housed for 2 days under continous light.

After these procedures, blood was collected from the animals' hearts, with all rats under pentobarbital 50 mg·kg^{-1} i.p. The blood samples were immediately centrifuged at 1000 g for 15 min and the serum was separated. Serum LH levels were assayed by radioimmunoassay using Rat LH Assay System (Amersham, Buckinghamshire, UK) whose sensitivity is more than 0.4 ng·ml^{-1}. Subsequently, all rats were quickly perfused through the heart with saline and a 4% paraformaldehyde solution (pH 7.4). The brains were quickly removed and immersed in the same fixative for 24 h. Serial frontal 50-μm sections were cut in a cryostat. Six sections around the anterior commissure and five sections on the median eminence were selected for immunohistochemistry. To demonstrate GnRH-like immunoreactivity, the avidin-biotin-peroxidase complex (ABC) method [2] was used as follows: A total of 11 sections in each rat were incubated with normal goat serum and a GnRH antiserum (diluted to 1:3000, UCB Bioproducts, Brussels, Belgium) for 3 days at 4°C, followed by incubation with biotinylated antirabbit IgG goat serum and ABC solution (Vectastain ABC kit, Vector Laboratories, Burlingame, CA, USA) at room temperature for 3 h each. GnRH-like immunoreactive neurons around the anterior commissure, and fibers and granules on the median eminence, appeared with 3,3′-diaminobenzidine tetrahydrochloride and hydrogen peroxide. The numbers of GnRH neurons in each rat were summed with all six sections around the anterior commissure in the hypothalamus. As for the median eminence, one section which showed the most GnRH fibers and granules was selected.

Data are presented as the mean ± SD. The results were statistically analyzed by one-way analysis of variance (ANOVA, Fisher PLSD, Stat View SE+, Abacus Concepts, Berkeley, CA, USA), and $P < 0.05$ was taken as the level of significance.

Results

In all rats, the operation and anesthesia were performed without complications. Bilateral palpebral ptosis and retraction were observed in the LS group during the 2-week study period.

Table 1 shows the results of the serum LH levels and the numbers of GnRH neurons around the anterior commissure. The difference in serum LH levels between the C and LS groups was not significant, but LH levels in the L group were significantly higher than those in the C and LS groups. The LC group showed very low values compared with the other groups. As for the GnRH neurons, although there were no significant differences among the C, LS, and LC groups, the L group showed significantly lower numbers than the LS or LC group.

GnRH fibers and granules in the median eminence of the C group seemed to resemble those of the LS group; however, those of the L group showed dense immunoreactivities compared with the C or LS groups (Fig. 1). The LC group showed thin immunoreactivities compared with the C group (Fig. 2).

Discussion

The present results demonstrate that continuous exposure to light decreases GnRH neurons in the hypothalamus, increases GnRH fibers and granules in the median eminence, and increases LH secretions. However, these histological and hormonal changes during continuous light conditions are suppressed by bilateral cervical sympathectomy or colchicine administration into the cerebral ventricle. Furthermore, the effects of cervical sympathectomy resemble that of colchicine administration. Colchicine suppresses the axonal transports of certain hypothalamic hormones, thereby increasing the number of neurons in the hypothalamus and decreasing secretion of the hormones in the median eminence [3].

Table 1. Serum LH level and the number of GnRH neurons around the anterior commissure in the hypothalamus

Group	LH ($n = 4$; ng·ml^{-1})	GnRH neurons ($n = 4$)
Control (C)	1.05 ± 0.17	242 ± 13
Light (L)	1.50 ± 0.22*	192 ± 26**
Light-sympathectomy (LS)	0.85 ± 0.29	256 ± 50
Light-colchicine (LC)	0.4 ($n = 2$), <0.4[a] ($n = 2$)	274 ± 52

GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone.
* $P < 0.01$ vs control or light-sympathectomy group.
** $P < 0.05$ vs light-sympathectomy or light-colchicine group.
[a] The sensitivity of this assay kit is more than 0.4 ng·ml^{-1}.

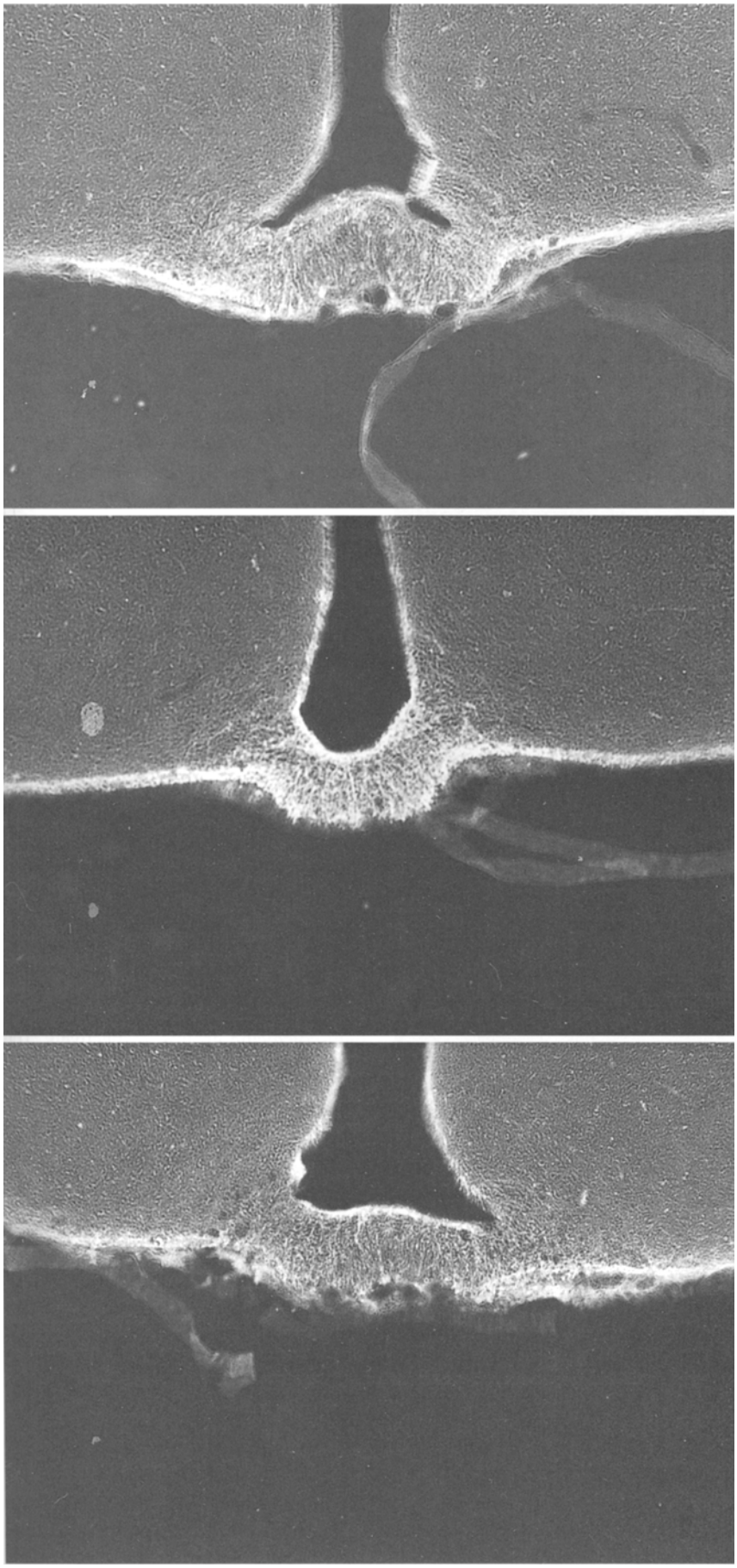

Fig. 1. Gonadotropin-releasing hormone (GnRH)-like immunoreactive fibers and granules in the median eminence. The *upper*, *middle*, and *lower* photomicrographs at ×20 are the control, light, and light-sympathectomy groups, respectively. The light group shows dense immunoreactivities compared with the control or light-sympathectomy group. The control group resembles the light-sympathectomy group

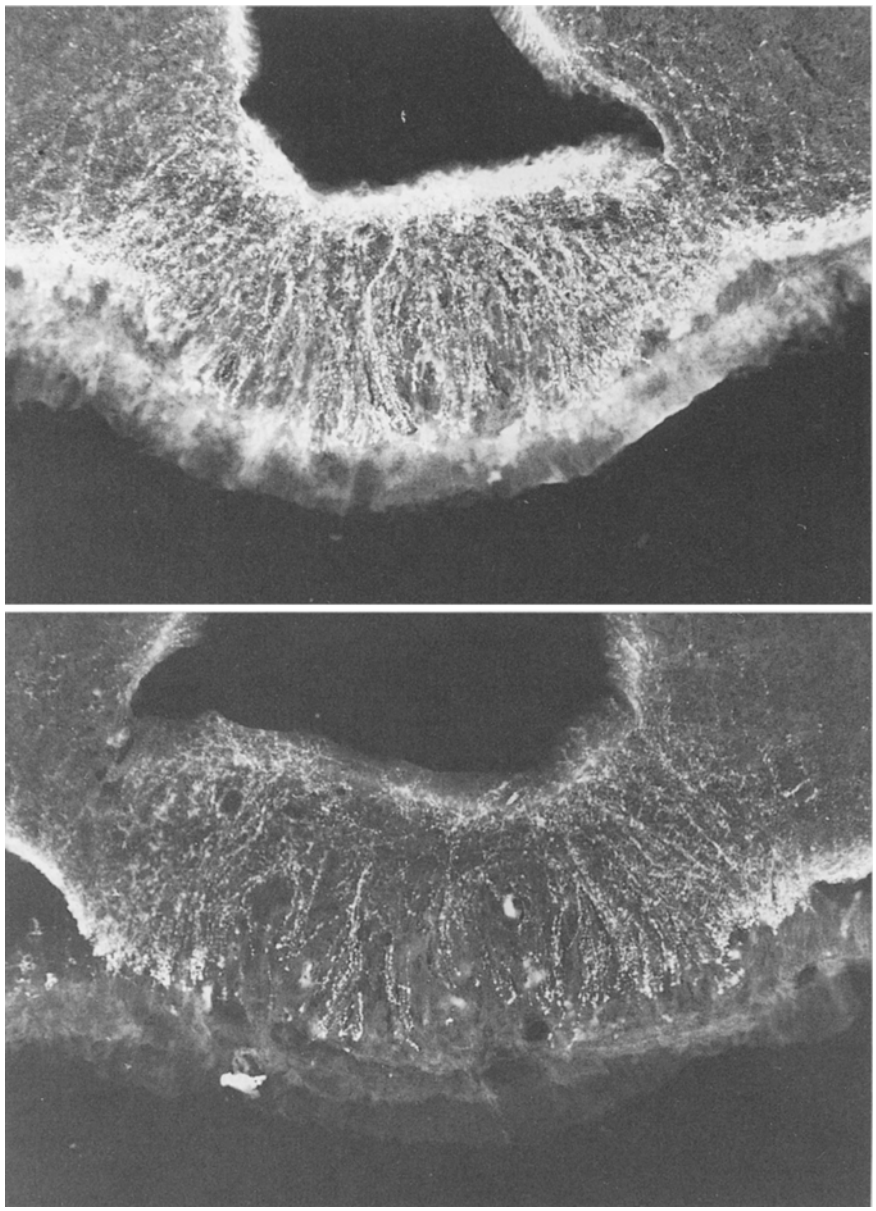

Fig. 2. Gonadotropine-releasing hormone (GnRH)-like immunoreactive fibers and granules in the median eminence at ×50. The *upper* and *lower* photomicrographs are the control and light-colchicine groups, respectively. The *lower* shows thin immunoreactivities compared with the *upper*

Therefore, the results imply that continuous exposure to light increases the axonal transports between the GnRH neurons and the median eminence, thereby decreasing the number of GnRH neurons in the hypothalamus and increasing the concentrations of GnRH fibers and granules in the median eminence. This is followed by an increase of LH secretion in the pituitary; however, cervical sympathectomy during continuous light conditions inhibits the increasing axonal transport of GnRH similar to the effects of colchicine administration. In the present study, cervical sympathectomy was performed with retention of the superior cervical ganglia, so that the postganglionic nerves would not be injured and norepinephrine (NE) would not be released in the nerve terminals. Decreasing GnRH secretion owing to transient NE release in the median eminence during the early phase of wallerian degeneration after superior cervical ganglionectomy has been recognized [4,5]; however, the present results are irrelevant to this matter. Consequently, the hypothesis that cervical sympathectomy during continuous exposure to light inhibits the increasing axonal transports of GnRH will probably be accepted.

In conclusion, the present study demonstrates that continuous exposure to light decreases the number of GnRH neurons in the hypothalamus, and increases GnRH fibers and granules in the median eminence, followed by an increase of LH secretion in the pituitary

in male rats. It also shows that bilateral cervical sympathectomy or colchicine administration into the cerebral ventricle under continuous light conditions inhibits these histological and hormonal changes. Therefore, we conclude that cervical sympathectomy may inhibit the axonal transport of GnRH between the GnRH neurons and the median eminence during continuous light conditions, because the response of cervical sympathectomy resembles that of colchicine administration.

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