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Cervical sympathectomy affects gonadotropin-releasing hormone, luteinizing hormone and testosterone in male rats

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Abstract: To examine the effects of bilateral cervical sympathectomy on the secretion of gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH) and testosterone (TS), 24 male rats were divided into four groups: control (C), light (L), sympathectomy (S), and light-sympathectomy (LS) groups. The C and S groups were kept under a 12-h light-dark cycle and the L and LS groups were kept under continuous light for 2 weeks. After 2 weeks, blood was collected and the rats were perfused with a fixative. GnRH neurons in the hypothalamus were stained immunohistochemically, and serum LH and TS levels were measured by radioimmunoassay. Although the difference in the number of GnRH neurons between the C and S groups was not significant, the L group was significantly lower than the C or LS groups. The serum LH and TS levels in the L group were higher than in the other groups. The present results suggest that continuous light increases GnRH secretion in the hypothalamus, followed by increased secretions of LH in the pituitary and TS in the testes, and bilateral cervical sympathectomy under continuous light inhibits these hormonal changes. However, a normal circadian rhythm does not affect gonadotropin secretion. Therefore, long-term and repeated stellate ganglion block may inhibit the increases of GnRH, LH, and TS secretions induced by continuous light.

Key words: Cervical sympathectomy, Stellate ganglion block, Gonadotropin, Testosterone, Rat

Introduction

Long-term and repeated stellate ganglion block (SGB) has been reported to be effective in some gynecological disease such as premenstrual tension syndrome or dysmenorrhea [1] while in rats superior cervical ganglionectomy decreases serum luteinizing hormone (LH) levels and depresses the postcastration rise in LH or follicle-stimulating hormone [2,3]. These findings imply that SGB affects the secretion of sex hormones, pituitary gonadotropic hormones, and hypothalamic gonadotropin-releasing hormone (GnRH). These hormones are also affected by environmental light and temperature in mammals [4–6]. In the present study, we performed bilateral cervical sympathectomy in male rats, regarded as a model of long-term and repeated SGB, and examined its effects on the secretion of GnRH, LH, and testosterone (TS) under a normal circadian rhythm and under continuous light.

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Materials and methods

Twenty-four adult male Wistar rats weighing 256.7 ± 23.4 g (mean ± SD) were used, and this study was conducted in accordance with the guidelines on animal experiments in Fukushima Medical College, the Japanese Government Animal Protection and Management Law (No. 105), and the Japanese Government Notification on Feeding and Safekeeping of Animals (No. 6). All rats were given access to food and water *ad libitum*.

The rats were randomly divided into four groups of six rats each: control (C), light (L), sympathectomy (S), and light-sympathectomy (LS) groups. The C and S groups were kept under a 12-h light-dark cycle from 7:00 to 19:00 at a room temperature of 24 °C for 2 weeks, and the L and LS groups were kept under continuous light and 24°C for the same period. Cervical sympathectomy was performed under pentobarbital 50 mg·kg^{-1} i.p. and atropine sulfate 0.05 mg·kg^{-1} i.m. The superior cervical ganglions were exposed through a ventral incision in the neck, and the bilateral inferior extremities of the superior cervical ganglion were cut off.

Two weeks later, blood was collected from the heart in all rats under pentobarbital 50 mg^{-1} i.p., and five rats

of each group were quickly perfused through the heart with saline and 4% paraformaldehyde solution (pH 7.4). Brains were quickly removed and immersed in the same fixative for 24 h. Serial frontal 50 μm sections were cut in a cryostat. Ten sections around the anterior commissure were selected for immunohistochemistry because most of the GnRH neurons exist between the medial septal area and medial preoptic area in the hypothalamus [7]. To demonstrate GnRH-like immunoreactivity, the avidin-biotin-peroxidase complex (ABC) method [8] was used as follows: Ten sections in each rat were incubated with normal goat serum and a GnRH antiserum (diluted to 1:3000, UCB Biopro, Bruxelles, Belgium) for 3 days at 4°C, followed by incubation with biotinylated anti-rabbit IgG goat serum and ABC solution (Vectastain ABC kit, Vector Laboratories Inc. CA, USA) at room temperature for 3 h each. GnRH-like immunoreactive neurons appeared with 3,3′-diaminobenzidine tetrahydrochloride and hydrogen peroxide. Six sections which showed the most GnRH neurons were selected, and the sum of GnRH neurons from all six sections was taken as the number of GnRH neurons in each rat.

The blood samples were immediately centrifuged at 1000 g for 15 min and the serum was separated. Serum LH and TS levels were assayed by radioimmunoassay (RIA). LH assay kits were provided by Amersham International (Rat LH assay system, Buckinghamshire, UK), and TS assay kits by Diagnostic Products (Total testosterone kit, Diagnostic Products Corporation, Los Angeles, CA, USA).

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 Inc., 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 S and LS groups during the 2-week study period (Fig. 1).

Most of the GnRH neurons were located around the anterior commissure between the medial septal area and the medial preoptic area in the hypothalamus (Fig. 2). There were 245.8 ± 13.6, 220.6 ± 30.9, 181.8 ± 32.7, and 267.8 ± 51.7 GnRH neurons in the C, S, L, and LS groups, respectively. The difference between the C and S groups was not significant, but the L group was significantly lower than the C or LS groups (Table 1).

The serum LH levels were 1.07 ± 0.23 ng·ml^{-1}, 1.18 + 0.20 ng·ml^{-1}, 1.52 ± 0.23 ng·ml^{-1}, and 0.87 ± 0.34 ng·ml^{-1} in the C, S, L, and LS groups, respectively. The difference between the C and S groups was not significant, but the L group was significantly higher than the other groups (Table 1).

The serum TS levels were 1.90 ± 1.37 ng·ml^{-1}, 1.78 ± 1.33 ng·ml^{-1}, 5.08 ± 2.79 ng·ml^{-1}, and 0.77 ± 0.88 ng·ml^{-1} in the C, S, L, and LS groups, respectively. The levels of TS in the L group was significantly higher than in the other groups (Table 1).

Discussion

In the present study, male rats were used to avoid the confounding influence of the pituitary sex cycle of gonadotropins. In the present study, bilateral cervical sympathectomy under normal circadian rhythm did not affect the number of GnRH neurons in the hypothalamus, nor did it affect the serum LH and TS levels. Although continuous light significantly decreased the number of GnRH neurons and increased serum LH and TS levels, sympathectomy under continuous light significantly inhibited these hormonal changes. In their morphological study, Shiotani et al. [7] demonstrated that a decrease in the number of GnRH neurons in the hypothalamus indicates increasing GnRH secretion and vice versa. Therefore, from the present morphological and hematological findings, it appears that continuous light increases GnRH secretion from the hypothalamus, followed by increases in LH secretion from the pituitary and TS from the testes, while sympathectomy only under continuous light suppresses these hormonal changes. However, under a normal circadian rhythm, sympathectomy does not affect the secretion of GnRH, LH, and TS.

In many animals, some of the light-induced stimulation descends to the lateral horn of the thoracic spinal cord via the supraoptic nucleus, and ascends through the cervical sympathetic trunk to the superior cervical ganglion (in which nerve conduction changes synapse) and reaches the pineal body, followed by decreasing melatonin secretion from the pineal body. In a dark environment, however, melatonin increases remarkably [7,9]. Especially in birds and mammals, melatonin it is now widely accepted that has an antigonadotropic action [4–6]. Although melatonin secretion exhibits a distinct circadian rhythm, Klein and Weller [10] reported that the rhythm was only abolished by continuous light, but during continuous dark it was maintained. The effect of cervical sympathectomy seems to resemble that of a continuous dark environment by cutting off the sympathetic nerve input to the pineal body due to light stimulation. Therefore, it appears that only continuous light increases gonadotropic secretion, and only under continuous light does cervical sympathectomy suppress these changes. However, under

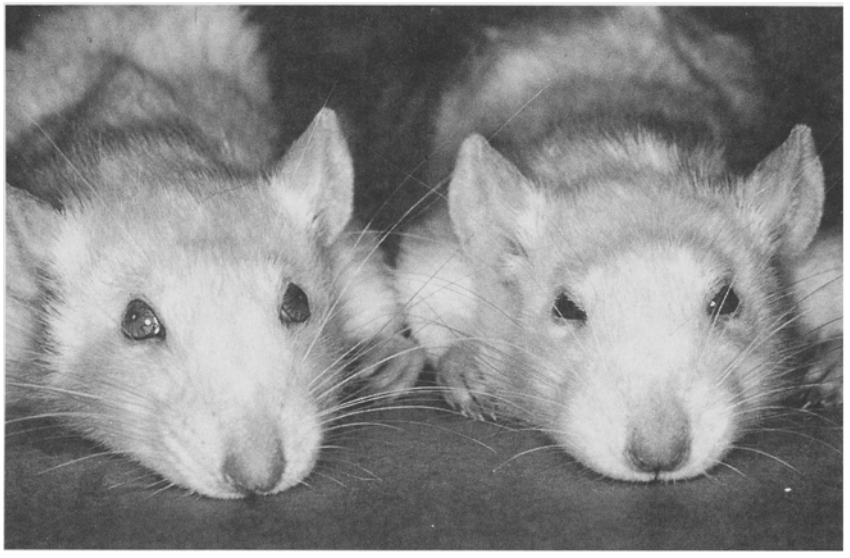

Fig. 1. *Right* rat after bilateral cervical sympathectomy showing bilateral palpebral ptosis and retraction compared with *left* normal rat

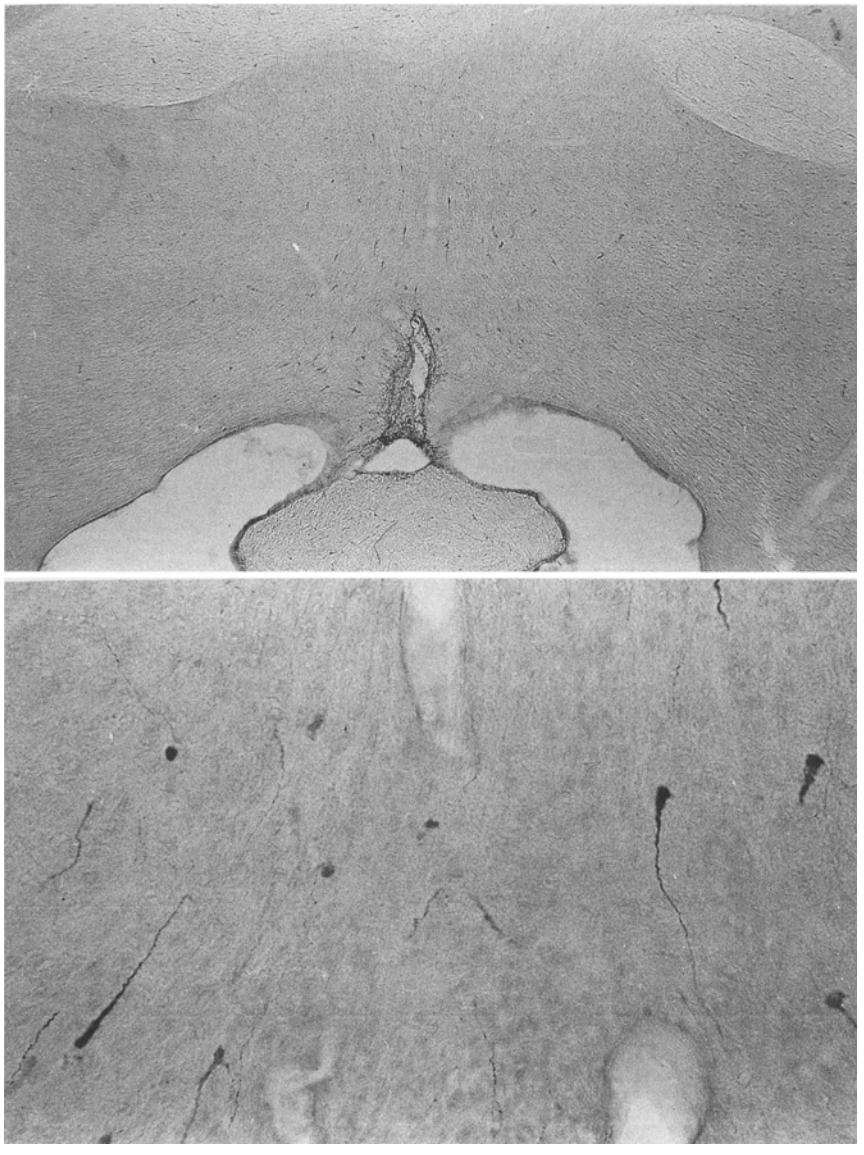

Fig. 2. Gonadotropin-releasing hormone (GnRH)-like immunoreactive neurons around the anterior commissure in the *upper* photomicrograph at a ×10. The *lower* photomicrograph shows the form of GnRH neurons at a ×50

Table 1. GnRH, serum LH and TS levels

Group	GnRH ($n = 5$)	LH ($n = 6$, ng·ml^{-1})	TS ($n = 6$, ng·ml^{-1})
Control	245.8 ± 13.6	1.07 ± 0.23	1.90 ± 1.37
Sympathectomy	220.6 ± 30.9	1.18 ± 0.20	1.78 ± 1.33
Light	181.8 ± 32.7[a]	1.52 ± 0.23[c]	5.08 ± 2.79[c]
Light-sympathectomy	267.8 ± 51.7[b]	0.87 ± 0.34[b]	0.77 ± 0.88

GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; TS, testosterone.
[a] $P < 0.05$ *s* control or light sympathectomy group.
[b] $P < 0.05$ *s* sympathectomy group.
[c] $P < 0.05$ *s* other groups.

normal circadian rhythm sympathectomy probably does not affect gonadotropic secretion. The present results also support this hypothesis. Little has been written about the relationship between superior cervical ganglionectomy and gonadotropins. Chiocchio et al. [2] and Romeo et al. [3] reported that superior cervical ganglionectomy depressed the postcastration rise of gonadotropins and decreased serum LH levels in male rats. They also demonstrated that superior cervical ganglionectomy resulted in a 40%–60% decrease in the norepinephrine content of the median eminence and in an increase of the GnRH content of the median eminence during wallerian degeneration. Although these studies reported that wallerian degeneration after superior cervical ganglionectomy decreased GnRH secretion from the hypothalamus, cervical sympathectomy in the present study did not appear to cause wallerian degeneration. Consequently, it is suggested that cervical sympathectomy mainly affects melatonin secretion in the pineal body and is not influenced by the norepinephrine content in the median eminence.

The effect of cervical sympathectomy in the rat is generally not equated with the effect of stellate ganglion block in humans; however, continuous palpebral ptosis observed by sympathectomy in the rat resembles human blepharoptosis found at stellate ganglion block. Thus, long-term and repeated SGB to humans may have the same effect as cervical sympathectomy in the rat. If so, SGB may inhibit the increases in the GnRH, LH, and TS secretions observed under continuous light but not under normal circadian rhythm.

In conclusion, the present study demonstrates that continuous light increases GnRH secretion in the hypothalamus, followed by increasing secretions of LH in the pituitary and TS in the testes in male rats, and also that bilateral cervical sympathectomy under continuous light inhibits these hormonal changes. However, under normal circadian rhythm it does not affect gonadotropin secretion. The mechanism of inhibition seems to act by increasing melatonin secretion due to suppression of sympathetic nerve conduction to the pineal body. Extrapolation of these findings to humans implies that long-term and repeated SGB would affect gonadotropins only under continuous light environment.

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