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Effects of Unilateral and Bilateral Orchidectomy on Laterality of Neurons of the Preoptic Area and Plasma Levels of Gonadotropins and Testosterone in Male Mice

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ABSTRACT—Possible involvement of direct neural connections between the testes and the hypothalamus in the neuroendocrine control of gonadal function was studied in male mice. Two-month-old male mice of the CD-1 strain were orchidectomized either unilaterally or bilaterally and changes in the size of neuronal nuclei and cell bodies in the medial preoptic area (POA) and plasma concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone were examined one week after operation. Both nuclei and cell bodies of POA neurons were significantly larger in the right side of the brain than in the left side. Either unilateral or bilateral orchidectomy failed to abolish the left-right difference. On the other hand, bilateral orchidectomy and removal of the left testis consistently caused a significant reduction in the size of neuronal nuclei on both sides of the POA, while removal of the right testis induced a significant decrease in the size of neuronal nuclei on the right side of the POA alone. By contrast, the cell body size of both sides of the POA was significantly larger in animals removed of their right testes than in those subjected to bilateral, left-sided or sham orchidectomy. Bilateral and unilateral orchidectomy caused a significant increase in plasma LH levels, but failed to show a significant elevation in plasma FSH levels. Removal of either side of the testis invariably caused an increase in plasma levels of testosterone. The present experiments clearly exhibited the left-right difference in the morphology of POA neurons in male mice. The results further suggest that the neural connections between the testes and the hypothalamus are involved in the control of hypophyseal-gonadal functions in male mice.

INTRODUCTION

Evidence has been accumulated on the presence of asymmetry in the brain of mammals. Diamond *et al.* [1] have reported that in young adult male rats specific regions in the right cerebral cortex are significantly thicker than the corresponding regions on the left. Asymmetry in oxygen consumption, glucose uptake and tissue contents of norepinephrine and dopamine in the cortex and other discrete areas of the brain is well known [2–4]. Several authors have also demonstrated the left-right difference in the endocrine hypothalamus in rats. Gerendai *et al.* [5] found that the content of gonadotropin-releasing hormone (GnRH) in the right side of the medial basal hypothalamus was significantly higher than in the left side in adult female rats. Bakalkin *et al.* [6] similarly reported that the content of luteinizing hormone-releasing hormone (LHRH) in the region of the arcuate nucleus (ARC) and the ventromedial nucleus (VMN) of the hypothalamus was greater in the right side than in the left side of the body of male rats. Fukuda *et al.* [7] further reported that the unilateral radio-frequency lesion to the right side of the medial anterior hypothalamus was effective in suppressing the ovarian compensatory hypertrophy in female rats. All these findings strongly

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suggest the existence of hypothalamic laterality in the regulation of gonadotropic functions in the rat. In this connection, Dörner and Staudt [8, 9] found that neither bilateral orchidectomy nor the replacement therapy with testosterone caused any changes in the morphology of neurons of the VMN and the preoptic area (POA) of the diencephalon in male rats. Although the POA is of principal importance in the control of gonadotropin secretion [10–12], morphological or functional laterality of this brain structure has not yet been known.

On the other hand, several authors have suggested that the neural mediation between the peripheral endocrine glands and the hypothalamus is involved in the regulation of neuroendocrine systems in rats [6, 13–17]. Gerendai and Halász [14] found that unilateral ovariectomy resulted in an enhanced uptake of amino acids into the contralateral ARC in rats. Bakalkin *et al.* [6] reported a decrease in LHRH contents in the hypothalamus contralateral to the side of unilateral orchidectomy in rats. They suggested that the neural signal from the gonads to the hypothalamus was transmitted through crossed afferent nerves. On the contrary, Mizunuma *et al.* [17] reported that the right-sided orchidectomy caused an increase in LHRH content in the ipsilateral hypothalamus in rats.

In the present experiments, in order to elucidate the laterality of the POA, male mice were orchidectomized either unilaterally or bilaterally and morphometrical changes in neurons of the POA and changes in plasma concentrations of gonadotropins and testosterone were investigated.

MATERIALS AND METHODS

Male mice of the CD-1 strain were used in the present study. They were housed in a temperature-controlled ($22 \pm 1°C$) room under 12-hr light (0600–1800) and 12-hr dark cycle with free access to laboratory chow and tap water. At 60 days of age, a total of 32 male mice were divided into four groups; 8 animals each of the first two groups were given removal of the testis of either the left or the right side (groups L and R) and 8 mice of the third group were orchidectomized bilaterally (group B). Eight mice of the last group served as controls receiving operations of abdominal incision but their testes were not removed (group S). All the above operations were done under ether anesthesia from 0900 to 1100 in the morning. One week after operation, animals were weighed and immobilized. Blood was collected from the jugular vein into the heparinized syringe. The plasma was separated by centrifugation at 3000 rpm for 30 min at 4°C and stored at $-20°C$ until assayed for gonadotropins and testosterone. Immediately after the blood collection, brains were quickly removed and fixed in 10% formalin. Prostates, preputial glands and testes, if remaining, were also taken out, weighed and fixed in 10% formalin. Samplings of blood and tissues were done from 0900 to 1100 in the morning.

Blocks of brain tissues containing the POA were embedded in paraplast. Sections were cut at 6 μm in thickness and stained with thionin. In each animal, the sizes of neuronal nuclei and cell bodies were measured in 50 cells each in both sides of the POA. The maximum diameter (a) and the diameter perpendicular to it (b) of individual neuronal nuclei were measured under the microscope using a micrometer, and the area of neuronal nuclei was calculated according to the formula of the ellipsoid; $A = \pi ab/4$. Profiles of neurons whose nuclear diameters were measured were traced using a camera lucida and the sizes of cell bodies of these neurons were measured with the aid of a tablet digitizer (MGC-1000, Mutoh, Japan). Neurons thus measured were located in the central region of the POA confined vertically between the anterior commissure and the optic chiasm, and laterally between two perpendicular lines drawn to the inner margins of the right and the left bed nuclei of the anterior commissure.

Plasma gonadotropins were measured by radioimmunoassay using a double antibody method [18, 19]. Highly purified luteininzing hormone (LH; NIADDK- rat LH-I-5) and follicle stimulating hormone (FSH; NIADDK-rat FSH-I-4) were radioiodinated with ^{131}I ($Na^{131}I$, Radiochemical Centre, Amersham) in the presence of lactoperoxidase and hydrogen peroxide using the method described previously [20] for the assay of plasma LH and FSH. Concentrations of LH in 100 μl plasma and FSH in 50 μl plasma were expressed in terms of ng NIADDK-rat LH-RP-2 and NIADDK-rat FSH-RP-2 per ml, respectively. Gonadotropins and antisera were kindly provided by NIADDK Rat Pituitary Hormone Distribution Program, NIH, Bethesda. We have previously confirmed that the rat assay system is satisfactory for the radioimmunoassay of LH and FSH in mouse plasma samples [21]. Plasma concentrations of testosterone were determined by radioimmunoassay using an antiserum to testosterone (HB-31, Teikoku Hormone MFG.) and [1, 2, 6, 7-^{3}H] testosterone (Radiochemical Center, Amersham) and expressed in terms of ng per ml plasma. Details of the radioimmunoassay of testosterone were described elslewhere [22].

Statistical analyses were performed by ANOVA and two-tailed Student's *t* test, or by Kruskal-Wallis test followed by Mann-Whitney's *U* test for multiple groups.

RESULTS

Morphometrical changes in neurons of the POA following unilateral and bilateral orchidectomy

Figure 1 shows the results of morphometry of POA neurons. The neuronal nuclei were significantly larger in the right POA than in the left ($P<0.001$ in group S). The neuronal nuclear size of the right POA was consistently greater than that of the left POA following left or right testis removal or bilateral orchidectomy ($P<0.01$ for groups L and R, and $P<0.001$ for group B, respectively). The cell bodies of neurons in the POA were similarly larger in the right side than in the left side, the difference between the left and right POA being statistically significant in all the groups S, R and B ($P<0.001$, in all comparisons).

On the other hand, either bilateral or unilateral orchidectomy rendered the neuronal nuclei significantly smaller in both the right and left sides of the POA ($P<0.001$ for either side of group B and for the right side of groups L and R, and $P<0.005$ for the left side of group L, as compared to the corresponding side of group S). Although statistically not significant, the nuclei of POA neurons appeared to be larger in right-testis-removed animals than in left-testis-removed mice. There were no significant differences in the size of cell bodies

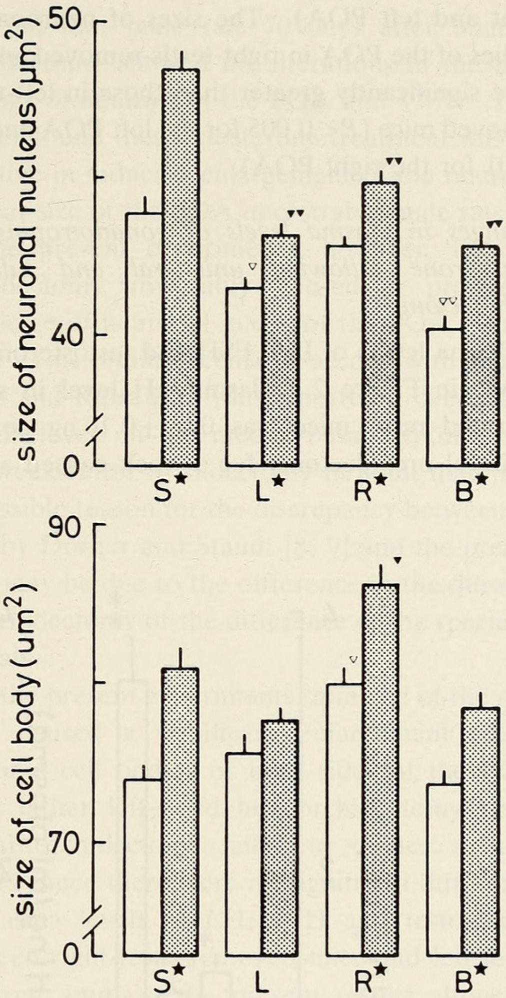

FIG. 1. Changes in the size of nuclei (upper panel) and cell bodies (lower panel) of POA neurons after unilateral and bilateral orchidectomy in mice. S: sham-orchidectomized animals, L: animals subjected to left-sided orchidectomy, R: animals subjected to right-sided orchidectomy, B: bilaterally orchidectomized animals. Open columns represent the left POA neurons and hatched columns show the right POA neurons. The number of animals used is 8 in each group. Asterisks exhibit that the difference between the left and the right sides of the POA is statistically significant ($P<0.001$ for double asterisks and $P<0.01$ for single asterisk). Triangles represent the statistically significant deviations from the corresponding side of the POA in group S ($P<0.001$ for double triangles and $P<0.005$ for single triangle).

of POA neurons among groups of sham-orchidectomy, left-sided hemiorchidectomy and bilateral orchidectomy. However, the cell bodies in either side of POA neurons of right-testis-removed animals were significantly larger than those of sham-operated animals ($P<0.005$ for the right and left POA). The sizes of neuronal cell bodies of the POA in right-testis-removed animals were significantly greater than those in left-testis-removed mice ($P<0.005$ for the left POA and $P<0.001$ for the right POA).

Changes in plasma levels of gonadotropins and testosterone following unilateral and bilateral orchidectomy

Plasma levels of LH, FSH and testosterone are shown in Figure 2. Plasma LH level in sham-operated male mice was 0.69±0.10 ng per ml. Bilateral orchidectomy for a week caused a five-fold increment in plasma LH concentrations ($P<0.001$). Unilateral removal of the left or the right testis also induced a significant increase in plasma LH levels ($P<0.005$ for the left and right testis removal). On the other hand, plasma FSH level was 24.9±2.8 ng per ml in sham-operated controls. No significant changes were detected in plasma FSH levels one week after unilateral or bilateral orchidectomy.

Plasma testosterone level in sham-operated control mice was 1.10±0.26 ng per ml. It slightly reduced after bilateral orchidectomy for a week, but the difference from the level of sham-operated

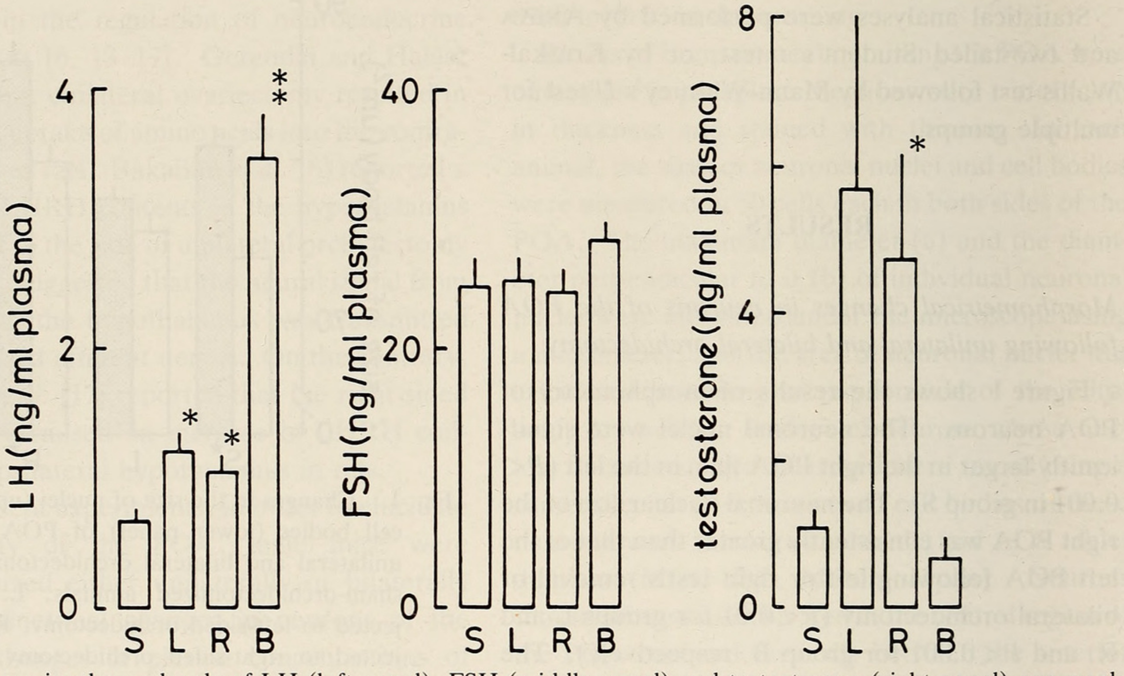

FIG. 2. Changes in plasma levels of LH (left panel), FSH (middle panel) and testosterone (right panel) one week after unilateral and bilateral orchidectomy in mice. Difference from the control value (S): $P<0.001$ for double asterisks and $P<0.005$ for single asterisk. See legend of Fig. 1 for abbreviations of S, L, R and B.

TABLE 1. Effects of unilateral and bilateral orchidectomy on the weight of preputial gland, prostate and the remaining testis in mice

group	no. of mice	organ weight (mg/50 g B.W.)			
		testis left	testis right	preputial gland	prostate
S	8	141.5±7.0	143.4±7.1	150.0±25.2	9.8±0.9
L	8	139.8±6.0*	146.4±8.0	134.9±21.6	11.7±0.9
R	8	135.6±5.4	137.5±7.5*	128.0±10.3	10.0±1.1
B	8	148.7±8.8*	147.0±6.6*	100.4±13.9	9.6±0.9

S: Sham-orchidectomy, L: left-sided orchidectomy, R: right-sided orchidectomy, B: bilateral orchidectomy.
* weight at orchidectomy.

controls was statistically not significant. In contrast, unilateral orchidectomy at either side caused an increase in plasma testosterone levels. The difference between right-testis-removed animals and sham-operated animals was statistically significant ($P<0.005$).

There were no significant differences between the weight of right testes and the left ones at the time of hemiorchidectomy (Table 1). One week after hemiorchidectomy, the weight of the remaining testis was not significantly different from the initial value of the contralateral testis. In the comparison of the weights of prostates and preputial glands among the four groups, preputial glands of bilaterally orchidectomized animals were, although statistically not significant, smaller than those of other groups.

DISCUSSION

The present experiments clearly demonstrated that there is an obvious left-right difference in the morphology of neurons of the POA. The size of neuronal nuclei of the POA was significantly larger in the right side than the left in male mice. In this connection, Gerendai *et al.* [5] and Bakalkin *et al.* [6] have already reported that the content of GnRH is greater in the right side of the hypothalamus than in the left side in male and female rats. Fukuda *et al.* [7] found that the unilateral lesion to the right side, but not to the left side, of the medial anterior hypothalamus effectively suppressed the ovarian compensatory hypertrophy in female rats and suggested that the right side of the medial anterior hypothalamus is indispensable for inducing the release of gonadotropins sufficient for the compensatory growth of the remaining ovary in hemiovariectomized rats. The present results are in good harmony with these previous findings and afford additional evidence for the hypothalamic laterality in the regulation of gonadotropin release in rodents. Since both the cell bodies and the nuclei of POA neurons are larger in size in the right side than in the left side, the neurons of the right POA may be playing more important roles than those of the left POA in the neuroendocrine control of gonadal function in mice.

On the other hand, Dörner and Staudt [8, 9] reported that male rats 70 days after bilateral orchidectomy showed no alterations in the morphology of neurons of the POA and VMN. They further found that testosterone treatment was not effective in inducing enlargement of the neuronal nuclear size of the POA in castrated male rats [8]. In the present experiments, however, bilateral orchidectomy invariably caused a prominent shrinkage of neuronal nuclei of the POA in male mice. This finding seems to accord with that of Kalra and Kalra [20] who reported a decrease in LHRH levels in the medial basal hypothalamus two weeks after orchidectomy in adult male rats. A possible reason for the discrepancy between the data by Dörner and Staudt [8, 9] and the present ones may be due to the difference in the duration of orchidectomy or the difference of the species of animals.

In the present experiments, removal of the right testis caused a significant enlargement of the neuronal cell bodies of both sides of the POA, while either left-sided hemiorchidectomy or bilateral orchidectomy failed to induce such an effect. Since there were no significant differences in plasma levels of LH, FSH and testosterone between right-testis-removed mice and left-testis-removed animals, the present results altogether suggest that the neural connections between the testes and the hypothalamus are possibly involved in the control of gonadal endocrine function in male mice.

Gerendai *et al.* [15] have already pointed out that unilateral adrenalectomy results in an enhanced synthesis of proteins in the VMN of the contralateral side in rats. Gerendai and Halász [14] similarly reported an accelerated incorporation of radioactive amino acids into the ARC contralateral to the side of ovariectomy. They suggested that the neural signal from the peripheral gland to the hypothalamus was probably transmitted via crossed afferent nerves. Bakalkin *et al.* [6] found that the left-sided hemiorchidectomy caused a decrease in LHRH contents in the right side of the hypothalamus and gave support to the crossed neural mediation between the testes and the hypothalamus. Mizunuma *et al.* [17], in contrast, reported that the right-sided orchidectomy caused an increase in LHRH content in the right hypothalamus in male rats. However, the present result showed no particular laterality in morphology of POA neurons after right-sided or left-sided orchidectomy in male mice. All these findings seem to indicate complicated features of neural influence from the peripheral endocrine organs to the hypothalamus.

It is generally believed that unilateral orchidectomy in rodents results in the attenuation in the negative feedback effect of androgens on the hypothalamo-pituitary system and consequently enhances the release of gonadotropins from the pituitary gland. However, several authors reported that plasma LH levels did not increase significantly but, instead, plasma FSH levels exhibited a substantial and prolonged increase after unilateral gonadectomy in rats [17, 24, 25]. Contrary to these results, Howland and Skinner [26] found that serum LH levels significantly increased on the next day of hemicastration and remained elevated for 40 days without any significant changes in serum FSH levels. Our results are consistent with those of Howland and Skinner [26]. In accordance with the elevated plasma LH levels in hemiorchidectomized mice, plasma levels of testosterone in these animals were higher than in the controls. Since it has been reported that plasma testosterone levels were either unchanged or lowered in hemiorchidectomized rats [27, 28], the present results further add the contradiction to the endocrine mechanism following unilateral gonadectomy.

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