Increased Incorporation of [^{3}H]-Lysine into Proteins of the Purkinje Cells of the Adult Male and Female Rat after Castration

Accumulating evidence indicates that the cerebellum is influenced by sex hormones[1–6]. The cerebellum of the rat accumulated injected ^{3}H-progesterone[6] as well as ^{3}H-estradiol[4], and pretreatment of the experimental animals with unlabeled testosterone inhibited the uptake of ^{3}H-estradiol by the cerebellum[4]. Progesterone implanted into the cerebellar cortex of ovariectomized rats increased pituitary luteinizing hormone and decreased hypothalamic luteinizing hormone-releasing factor[5]. RNA base ratios were altered in the cerebellum of the rabbit following ovariectomy and treatment with ovarian steroids[1], and the incorporation of ^{14}C-lysine into cerebellar proteins was inhibited following injection of pharmacologic amounts of estradiol to ovariectomized rats[3]. A recent quantitative autoradiographic study has shown that the incorporation of ^{3}H-lysine into neurons of the Purkinje, molecular, and granular layers of the cerebellar cortex did not fluctuate during the estrous cycle of the rat, but that incorporation is specifically inhibited in the Purkinje cells of anovulatory adult female rats treated neonatally with testosterone propionate[2].

These studies encouraged us to determine by quantitative autoradiography if castration of adult male and female rats is associated with changes in the incorporation of ^{3}H-lysine into the cell proteins of the 3 layers of the cerebellar cortex.

Methods. Male and female Holtzman rats (95 days old) bilaterally castrated 5 weeks previously and their intact controls were injected i.p. with 2 μCi/g body wt. of L-4,5-^{3}H(n)-lysine monohydrochloride (spec. act. = 7.5 Ci/mmol, Amersham/Searle), according to the procedures previously described in detail[7–9]. 30 min later the rats were perfused with a formalin solution and the brains processed for autoradiography[7–9]. 6 μm coronal sections of the cerebellum were prepared from paraplast embedded brain. After deparaffinization, the sections were coated with Kodak NTB 2, exposed for 7 weeks, developed, and finally stained with methylgreen pyronin.

Silver grains were recorded by the same investigator at 1600 × for the granule cells, the Purkinje cells, and the stellate cells of the molecular layer for each group of 5 or 6 rats. Grains were counted in all possible focal planes from at least 250 neurons (50 neurons/rat) for each cell type (Table.) Background fog in the autoradiograms was insignificant, i.e., 0–2 grains in cerebellar neurons from rats not injected with ^{3}H-lysine. Significance between the intact and castrated groups of rats was determined by Student's *t*-test.

Results and discussion. Castration of the adult male or female rat for a period of 5 weeks is followed by a significant and specific increase in the incorporation of ^{3}H-lysine into proteins of the Purkinje cells (Table). Incorporation into the stellate cells of the molecular layer and granule cells of the internal granular layer was not altered following castration of male or female rats (Table). We have previously reported that the incorporation of ^{3}H-lysine into neurons of the 3 layers of the cerebellar cortex was unchanged during the various stages of the rat estrous cycle[2]. Therefore, data obtained from the ovariectomized rats could be compared to that occurring during the proestrus stage of the cycle (Table).

In previous reports, the ability of specific hypothalamic nuclei of both male and female rats to respond to castration with an increase in incorporation of ^{3}H-lysine into proteins suggested that these neurons represent feedback sites for the sex steroids or their metabolites[7,9]. The specific and similar pattern of response of the Purkinje cells of the cerebellar cortex to castration also supports this interpretation. Additionally, the Purkinje cells[2] as well as specific hypothalamic nuclei of the adult female rat[8] injected neonatally with testosterone propionate demostrated an inhibition in the incorporation of ^{3}H-lysine into proteins.

To our knowledge, the parallel responses for both the Purkinje cells and specific hypothalamic nuclei to the differing endocrine manipulations[2,7–9] is the first evidence that the previously considered 'asexual' Purkinje cell is sensitive to the sex hormones. Neither previous research[1–6], nor the data presented here are sufficient to establish clearly the nature of the relationship.

Résumé. La castration d'un mâle adulte ou d'une femelle de rat est suivie après 5 semaines d'une augmentation significative de l'incorporation de la ^{3}H-lysine dans les protéines des cellules de Purkinje.

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Silver grains per neuron in the 3 layers of the cerebellar cortex of intact and castrated male and female rats

Group	Silver grains per neuron		
	Purkinje	Granule	Molecular
Intact males	63.45 ± 1.26[a]	5.89 ± 0.15	11.08 ± 0.24
Castrated males	71.16 ± 2.06[a] $P < 0.005$	5.79 ± 0.22 NS[b]	10.98 ± 0.43 NS
Intact females (proestrus)	56.39 ± 1.14[c]	5.03 ± 0.12	10.31 ± 0.33
Castrated females	68.41 ± 1.12[a] $P < 0.001$	4.95 ± 0.14 NS	10.10 ± 0.40 NS

[a] Mean ± standard error; the mean is the average value of 5 brains. [b] *P* is not significant. [c] Mean ± standard error; the mean is the average value of 6 brains.

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