Age-Related Differences in the Effect of Castration upon Hypothalamic LHRC Content in Male Rats

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Abstract. Male rats aged between 15 and 75 days were orchidectomized or only anesthetized. After various periods from 2 to 42 days, 5 animals of both groups were sacrificed. Serum concentrations of lutestinizing hormone (LH) and follicle-stimulating hormone (FSH) as well as the hypothalamic luteinizing hormone-releasing hormone (LHRC) content were determined by radioimmunoassay. At the times studied within 15 days of castration, no significant change in hypothalamic LHRC content was observed in rats orchidectomized at 21 days of age. However, when studied 3-6 weeks after castration, those animals showed a reduced hypothalamic LHRC content. No differences were observed between those orchidectomized rats aged 15, 18 or 21 days and studied 1 week later. In contrast, castration of 75-day-old rats resulted in a significant reduction of the hypothalamic LHRC content after 2-15 days. A significant decrease was also observed 1 week after orchidectomy of rats aged 24-50 days. 1 day after orchidectomy, LH and FSH serum levels were markedly increased in 21- and 24-day-old rats. In the latter, serum LH concentrations did not change any further whereas in the former a plateau was only seen after 1 week. In rats orchidectomized at various ages ranging from 15 to 50 days, no consistent differences appeared in serum gonadotropin concentrations evaluated 7 days later. According to these data, variations in hypothalamic LHRC content after orchidectomy differ according to age and maturity. In 15- to 21-day-old rats, the age-related increase in hypothalamic LHRC content was not immediately affected by castration as it was in older animals. This might suggest that (1) changes in sensitivity to gonadal factors according to age are primarily localized in the hypothalamus, (2) some maturational processes in the hypothalamus are not dependent on the presence of gonadal factors.

The role of gonadal factors in triggering hypothalamic-pituitary maturation at puberty is still a matter of controversy. The age-related variations in gonadotropin circulating levels described by Conte et al. [10] in patients with gonadal dysgenesis would seem to argue against a necessary role of the human gonads in the hypothalamic-pituitary maturation.

In order to test this hypothesis in the rat, the effects of castration on plasma gonadotropin levels were analyzed in relation to age. No conclusion can be drawn from these studies since the rise in luteinizing hormone (LH), 2-5 days after orchidectomy, of adult male rats, has been found to be either higher or similar to that seen in immature orchidectomized animals [18, 26, 27, 29]. In immature rats, very little is known about the variations of the hypothalamic luteinizing hormone-releasing hormone (LHRC) content after castration. This contrasts with the extensive demonstration of a depletion in hypothalamic LHRC following castration of adult rats [1, 2, 6, 8, 9, 17, 19-22, 30, 32, 35, 37]. Only one investigation has been performed in immature rats [3]: ovariectomy at 28 days of age resulted, 5-20 weeks later, in the suppression of the increase in hypothalamic LHRC normally seen throughout puberty.

Thus, the aim of this work is to provide additional data on the age-related variations in hypothalamic LHRC and serum gonadotropin levels after castration of male rats in order to investigate the role of the gonads in the control of the hypothalamic-pituitary maturation.

Materials and Methods

Rats

Wean strain male rats were studied either before and around (onset of puberty (15, 18, 21, 24, 27 and 30 days old), at the time of puberty (50 days old) or at the adult stage (75 days old). They were housed under standardized conditions of temperature (24-26°C) and light/darkness rhythm (14/10 h), with free access to food and water. In rats of each age group, a bilateral orchidectomy was performed by surgical castration under light ether anesthesia between 10 and 12 h. An equal number of intact rats was sham-orchidectomized at the same time, but were not operated upon.

Experimental Protocols

A first experiment was designed to evaluate variations in hypothalamic prolactin function according to age. Wean animals were housed and treated as in the orchidectomized rats group, and castrated at 21 or 75 days. A second experiment was carried out to determine variations in response to age in rats orchidectomized between 15 and 50 days and studied 1 week later. These experiments took place in December, March and July, and were appropriate time according to the seasonal variations in pituitary function. The results from the orchidectomized were as well as from the sham-orchidectomized group, were weighted and analyzed under light ether anesthesia. Decapsulation was always performed between 14 and 16 h; trunk blood was collected immediately and stored overnight at 4°C before being centrifuged. Serum was separated and stored frozen. In rats of both groups, seminal vesicles were removed through an abdominal incision whereas testes were also obtained in intact animals. These organs were kept in physiological saline before being weighed. Finally, the hypothalamus was dissected from the anterior border of the meninges body to a transversal cut rostrally, 3 mm in front of the optic chiasma in order to include the preoptic area in the tissue fragment. Sections were made along the lateral hypothalamic sulci. A frontal cut was performed at 2-3 mm depth. The hypothalamus was immediately removed and immersed in 1 ml ice-cooled 2 M acetic acid, pH 2.3.

Extraction and Radioimmunoassay of Hypothalamic LHRC

Hypothalamic tissue was homogenized by ultrasonication in 1 ml acetic acid. After neutralization using 13.4 ml of NH4OH, pH 13.6, and centrifugation at 4°C, the supernatant containing the extracted LHRC was separated and prepared at serial dilutions in the assay buffer (phosphate-buffered saline, pH 7.5, containing 10 mmol/L of sodium azide, 1 g/L). The mean yield of this extraction procedure (± 1 SD) was 73.4% as measured using several synthetic LHRC concentrations from 10 to 1 mg/L. The stability of LHRC extracted from hypothalamus and the reproducibility of its radioimmunoassay was determined by measuring at 6-month intervals the immunoreactivity contained in 29 extracts of rat hypothalami: a significant linear correlation was obtained (y = 0.87 x - 0.34, r = 0.83, p < 0.001).

The radioimmunoassay of LHRC in hypothalamic extracts was performed in duplicate using a double antibody method. The anti-LHRC antibody was the highly specific RR-5 antisemum generously provided by Drs. Root and Reiter. It was used at a final dilution of 1:100,000. The specificity of this antisemum for the intact decaperea has previously been demonstrated [11]. Radiolabeled LHRC used was either prepared in the laboratory [4] or obtained from a commercial source (New England Nuclear, Boston, Mass.). The specific activity of both materials varied from 200 to 500 mCi/mg. Following a preincubation of 16 h, the tracer was added for 24 h at 4°C. The separation of free from bound radioactivity was obtained for 1 h in the presence of a second antibody raised against rabbit y-globulin and coupled to cellulose according to the technique of Wiede and Porth[4] (Institut National des Radioéléments, CEA, France). Non-specific binding was less than 5% of the total radioactivity. The sensitivity was around 1 pg/tube. The intra-assay coefficient of variation was 18.2%.

The standard curves were not different when prepared either in the assay buffer or in the presence of the currently used dilutions (1/400 to 1/20 of the extraction medium: acetic acid and NH4OH).

Characterization of LHRC in Hypothalamic Extracts

The immunoreactive material extracted from rat hypothalamus has previously been characterized by chromatography on carboxymethyl cellulose [5]. The elution pattern was similar to that of 1-10 LHRC. In addition, inhibition curves obtained with serial dilutions of hypothalamic extracts from immature as well as adult male rats showed a parallelism with the standard curve in a log-log system. Finally, the bioactivity of hypothalamic extracts prepared from hypothalami of 21- and 50-day-old rats was investigated in vitro using monolayer cultures of pituitary cells prepared as already described [12]. Increasing concentrations of hypothalamic extracts resulted in a dose-related rise in follicle-stimulating hormone (FSH) and LH release which occurred similarly at both ages.

Gonadotropin Radioimmunoassays

Serum concentrations of FSH and LH were determined by double antibody radioimmunoassays in duplicate, using NIH-MDD pituitary gonadotropin reagents kindly supplied by the pituitary agency of NIH. These methods have previously been described [13]. The limits of sensitivity are 1 and 5 ng/tube for LH and FSH, respectively. Since LH and FSH were measured, respectively, in 0.3 and 0.1 ml serum, detection limits were 3 and 50 ng/ml rat serum. Results are expressed with reference to the NIH-MDD HR-1 standard preparation.

Statistical Analysis

Since the biological parameters studied appeared to have a log-normal distribution, the mean values and standard deviations were calculated after logarithmic transformation. The significance of differences between geometric means obtained by that calculation was determined using the unpaired Student's t test.

Results

Growth Parameters (fig. 1)

After castration at 21 days, the growth velocity, as compared to that of intact rats, decreased, the mean body weight being significantly lower in orchidectomized than in sham-orchidectomized rats aged 44, 57 and 63 days (fig. 1). 2 days after orchidectomy, mean weight of seminal vesicles corrected for total body weight was similar in both sham-orchidectomized and orchidectomized rats. However, from the age of 30 days onwards, i.e. 9 days after castration, a significant difference was observed and became more marked with age.

Hypothalamic LHRC Content in Relation to Time after Castration (fig. 2)

In figure 2, the geometric means ± SEM of the hypothalamic LHRC content are represented in relation to time after sham anesthesia or orchidectomy performed either at 21 days or at 75 days of age. Between 2 h and 2 days after castration, similar values were observed in sham-orcheste-
tized and orchidectomized animals of the immature group. However, 2 days after castration, adult rats showed a significant decrease in the LHHRH content. From 2 to 15 days after orchidectomy, this reduction became more marked in adult orchidectomized animals. In contrast, an age-related increase in the LHHRH content occurred similarly in rats orchidectomized before puberty as in controls. From 3 to 6 weeks after castration of 21-day-old rats, the hypothalamic LHHRH content was found to be significantly lowered, its level being similar to that observed 15 days after castration in adult rats.

**Serum Gonadotropin Concentrations in Relation to Time after Castration (Fig. 3)**

After castration of 21- and 75-day-old rats (Fig. 3), serum levels of LH and FSH showed a highly significant increase during the first 24-hour period. After 2 days, no further changes were observed for both gonadotropins in adult castrates and for FSH in immature rats. As shown in Figure 3, a lower mean value of serum FSH concentration was obtained 3 weeks after castration of 21-day-old rats. However, by repeating this experiment, no different results were obtained according to time after castration. In immature castrates, LH serum levels showed a 2- to 3-fold increase between 2 days and 1-2 weeks following orchidectomy, thus taking more time than in adult rats to reach a plateau.

**Hypothalamic LHHRH Content and Serum Gonadotropin Levels in Relation to Age at Castration (Fig. 4)**

In Figure 4, the results are represented that were obtained in rats orchidectomized at various ages ranging from 15 to 50 days and studied after a 7-day period. At that time, a highly significant increase in LH and FSH serum concentrations was observed in all groups without any obvious difference according to age at castration. In contrast, hypothalamic LHHRH content did not change significantly in rats castrated between 15 and 21 days of age although a significant decrease was observed in rats aged 24 days and more.

**Discussion**

We have shown that the effects of castration upon the hypothalamic LHHRH content are different according to age and maturity of the male rat. In immature animals aged between 15 and 21 days, the content of LHHRH in the hypothalamus is not immediately modified following castration whereas it is reduced later on. Such a decrease occurs earlier in rats orchidectomized after the age of 21 days.

These data provide indirect evidence that hypothalamic sensitivity to gonadal hormones changes with age. This hypothesis is in agreement with the observation that pituitary gonadotropins escape the inhibitory control exerted by estradiol in estradiol-treated orchidectomized rats attaining puberty [33]. However, the determination of gonadotropin rise after castration does not provide a reliable model for studying the changes in hypothalampituitary sensitivity with age in rats [27, 29]. After neonatal ovariectomy or orchidectomy, no consistent changes in gonadotropin secretion with age have been observed [13, 23]. In contrast, we conclude that the measurement of hypothalamic LHHRH content may be useful to define the maturational status of the hypothalamus.

In addition, our data suggest that, throughout maturation, the increase in hypothalamic LHHRH content [1, 7, 34]
does not depend, at least initially, on the presence of gonadal factors. To our knowledge, the only previous study dealing with this response in immature castrated rats has been performed in female rats and started 3 weeks after ovariectomy [3]. Therefore, it is possible that an early increase in the hypothalamic LHRR content has been missed by these authors.

This emphasizes possible variations in the hypothalamic control of gonadotropin secretion according to time elapsed following castration. This concept is supported by other findings: the release of LHRR estimated in vivo by sampling portal blood or by cannulation of the median eminence area has been found to be increased 4 days or 8 weeks after castration [14, 31], but decreased in long-term (3 months) ovariectomized rats [24, 35]. In addition, the study of orchidectomized monkeys at different times after castration has evidenced marked changes in frequency and amplitude of the pulsatile pattern of LH release, which is likely to be determined by the hypothalamus [20].

Our data also show that the postcastration rise in LH differs according to time in immature and adult rats: the plateau level is observed earlier in the latter than in the former. This may account for the discrepancies between reports on the LH response to castration according to age [18, 26, 27, 29]. 1 week after orchidectomy, gonadotropins were at a plateau level in both 21- and 75-day-old rats whereas hypothalamic LHRR content was obviously lower in 21-day-old rats than in mature animals. It is possible that the age-related differences in response to castration were studied 7 days after the operation.

In immature rats orchidectomized at 21 days of age, the hypothalamic LHRR content increased initially but decreased subsequently as it did earlier after castration in pubertal and adult rats. Thus, after a first period characterized by the increase in LHRR, it appears that the long-term preservation of the hypothalamic LHRR content in immature rats is determined by gonadal factors, as previously suggested by Barnea et al. [3]. Since early depletion of hypothalamic LHRR stores after castration in 21-day-old rats is known to persist for several months [2], we did not study these animals for more than 15 days after orchidectomy.

Finally, some discrepancies between the results obtained from different experiments in this study should be considered. For instance, in the hypothalamic FSH concentration measured 3 weeks after castration was found to be either at the plateau level or somewhat lower in two different experiments. The hypothalamic LHRR content measured in sham-sterilized animals was not higher at 51 days than at 42 days of age. These differences between experiments probably do not result from circadian variations since the different groups of animals were sacrificed at the same time of the day. Ultradian changes in hormone secretion may partly account for the observed differences [16]. Finally, circannual variations should also be considered since the data shown in this paper were obtained at different times of the year [25, 38].

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