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Age-Related Differences in the Effect of Castration upon Hypothalamic LHRH Content in Male Rats[1]

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Abstract. Male rats aged between 15 and 75 days were orchidectomized or only anesthetized. After various periods from 2 h to 42 days, 8 animals of both groups were sacrificed. Serum concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) as well as the hypothalamic luteinizing hormone-releasing hormone (LHRH) content were determined by radioimmunoassay. At the times studied within 15 days of castration, no significant change in hypothalamic LHRH 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 LHRH content. No differences were observed between anesthetized and 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 LHRH 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- as well as 75-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 LHRH content after orchidectomy differ according to age and maturity. In 15- to 21-day-old rats, the age-related increase in hypothalamic LHRH 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 hypothalamopituitary 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 hypothalamopituitary 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 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 (LHRH) content after castration. This contrasts with the extensive demonstration of a depletion in hypothalamic LHRH 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, 3–20 weeks later, in the suppression of the increase in hypothalamic LHRH normally seen throughout puberty.

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

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Materials and Methods

Rats

Wistar strain male rats were studied either before and around the 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 scrotal approach under light ether anesthesia between 10 and 12 h. An equal number of intact rats were sham-anesthetized at the same time, but were not operated upon.

Experimental Protocols

A first experiment was designed to evaluate variations in hypothalamopituitary function according to time after castration (from 2 h to 6 weeks) in rats orchidectomized at 21 or 75 days. A second experiment was carried out to determine variations according to age in rats orchidectomized between 15 and 50 days and studied 1 week later. Those experiments took place in December, March and July. At the appropriate time according to those protocols, 8 rats from the orchidectomized as well as from the sham-anesthetized groups were weighed and sacrificed under light ether anesthesia. Decapitation 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 weighing. Finally, the hypothalamus was dissected from the caudal border of the mamillary bodies 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 LHRH

Hypothalamic tissue was homogenized by ultrasonication in 1 ml acetic acid. After neutralization using 13.4 *M* NH_4OH, pH 13.6, and centrifugation at 4 °C, the supernatant containing the extracted LHRH was separated and prepared at serial dilutions in the assay buffer (phosphate-buffered saline, pH 7.5, enriched with gelatine, 1 g/l). The mean yield of this extraction procedure (± 1 SD) was 73 ± 4% as measured using several synthetic LHRH concentrations from 0.5 to 10 ng/tube. The stability of LHRH extracted from hypothalami and the reproducibility of its radioimmunoassay were determined by measuring at 6-month intervals the immunoreactivity contained in 29 extracts of rat hypothalami: a significant linear correlation was obtained ($y = 0.97 \times -0.34$, $r = 0.83$, $p < 0.001$).

The radioimmunoassay of LHRH in hypothalamic extracts was performed in duplicate using a double antibody method. The anti-LHRH antibody was the highly specific RR-5 antiserum generously provided by Drs. *Root* and *Reiter*. It was used at a final dilution of 1/100,000. The specificity of this antiserum for the intact decapeptide has previously been demonstrated [11]. Radioiodinated LHRH 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 μCi/μg. Following a preincubation of 18 h, the tracer was added for 24 h at 4 °C. The separation of free from bound radioactivity was obtained by stirring for 1 h in the presence of a second antibody raised against rabbit γ-globulins and coupled to cellulose according to the technique of *Wide and Porath* [36] (Institut National des Radio-éléments, Fleurus, Belgique). Nonspecific binding was less than 5% of the total radioactivity. The sensitivity was around 1 pg/tube. The inter-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 (1/4 to 1/20 of the extraction medium (acetic acid and NH_4OH).

Characterization of LHRH in Hypothalamic Extracts

The immunoreactive material extracted from rat hypothalami has previously been characterized by chromatography on carboxymethyl cellulose [5]. The elution pattern was similar to that of 1–10 LHRH. 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 logit-log system. Finally, the bioactivity of hypothalamic extracts prepared from hypothalami of 21- and 50-day-old rats has been 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 NIAMDD rat pituitary gonadotropin reagents kindly supplied by the pituitary agency of NIH. These methods have previously been described [15]. 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 NIAMDD IRP-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-anesthetized 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-anesthetized 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 LHRH Content in Relation to Time after Castration (fig. 2)

In figure 2, the geometric means ± SEM of the hypothalamic LHRH 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-anesthe-

tized and orchidectomized animals of the immature group. However, 2 days after castration, adult rats showed a significant decrease in the LHRH 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 LHRH 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 LHRH 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 LHRH 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

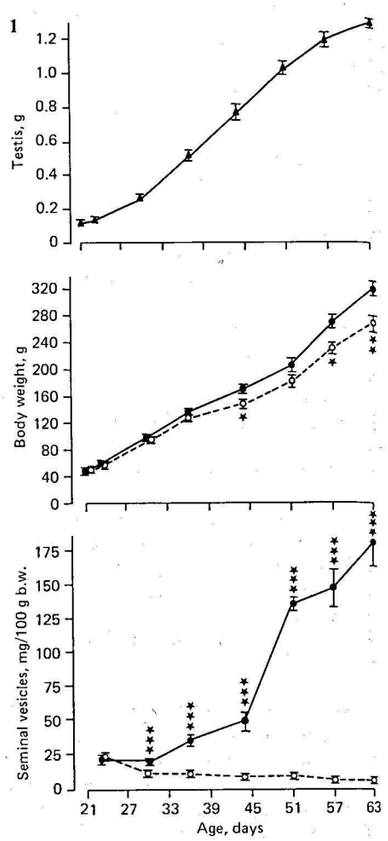

Fig. 1. Age-related variations of mean (± SEM) total body weight, testis weight and seminal vesicle weight in intact rats (●) or rats orchidectomized (○) at 21 days of age. Each group contained 8 animals. Stars indicate significance of differences between intact and castrated rats: *p<0.05, **p<0.005, ***p<0.001.

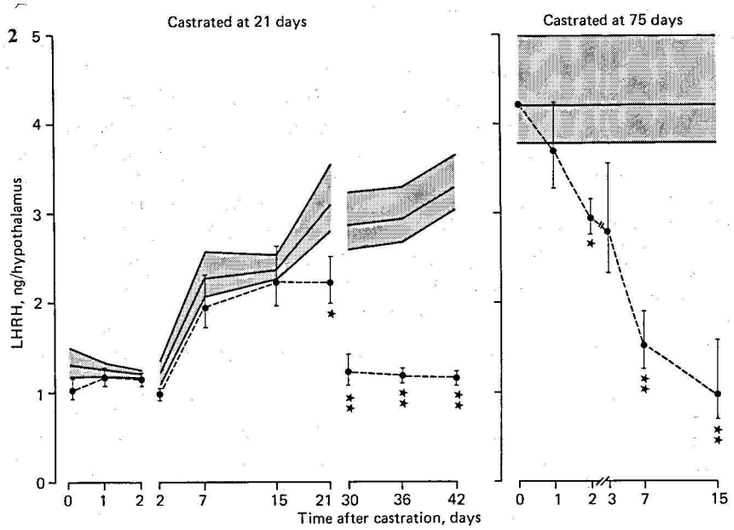

Fig. 2. Time-related variations in hypothalamic LHRH content after castration or sham anesthesia of immature (21-day-old) and adult (75-day-old) rats. In immature animals, the study consisted of three experiments according to time following orchidectomy: 2 h to 2 days, 2–21 days and 30–42 days. Shaded area = sham-anesthetized animals; ● = archidectomized rats. Significant differences between sham-anesthetized and orchidectomized rats are indicated by stars (* p < 0.05, ** p < 0.001). The geometric mean ± SEM of 8 rats is given.

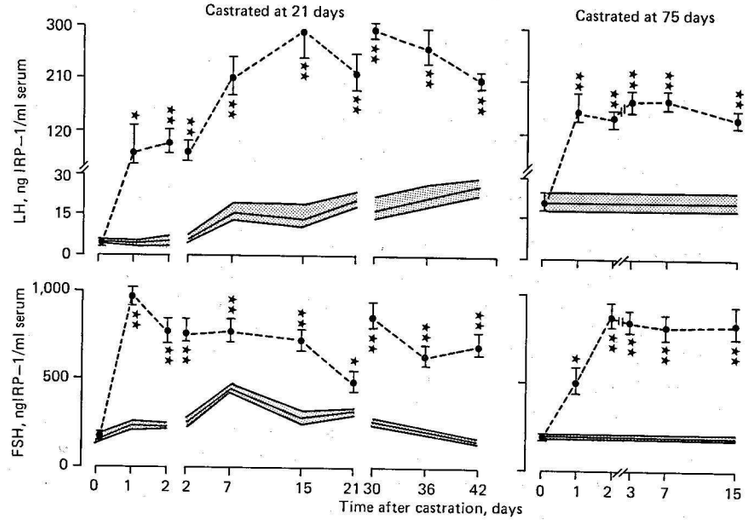

Fig. 3. Time-related variations of serum gonadotropin concentrations after castration or sham anesthesia of immature (21-day-old) and adult (75-day-old) rats. In immature animals, the study consisted of three experiments according to time following orchidectomy: 2 h to 2 days, 2–21 days and 30–42 days. Shaded area = sham-anesthetized animals; ● = orchidectomized rats. Significant differences between sham-anesthetized and orchidectomized rats are indicated by stars (* p < 0.005, ** p < 0.001). The geometric mean ± SEM of 8 rats is given.

was observed in all groups without any obvious difference according to age at castration. In contrast, hypothalamic LHRH 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 LHRH content are different according to age and maturity of the male rat. In immature animals aged between 15 and 21 days, the content of LHRH 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 ovariectomized rats attaining puberty [33]. However, the determination of gonadotropin rise after castration does not provide a reliable model for studying the changes in hypothalamopituitary 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 LHRH content may be useful to define the maturational status of the hypothalamus.

In addition, our data suggest that, throughout maturation, the increase in hypothalamic LHRH content [1, 7, 34]

Fig. 4. Age-related variations in hypothalamic LHRH content and serum gonadotropin levels determined 7 days after orchidectomy (▨) or sham anesthesia (▩) of rats aged 15–50 days. Significant differences between sham anesthetized and orchidectomized animals are indicated by stars (* p < 0.01, ** p < 0.001). The geometric mean ± SEM of 8 rats is given.

does not depend, at least initially, on the presence of gonadal factors. To our knowledge, the only previous study dealing with LHRH in the hypothalamus of 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 LHRH 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 LHRH 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 [28].

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 LHRH content was obviously lowered in adult castrates but increased normally in immature castrates. This is the reason why 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 LHRH content increased initially but decreased subsequently as it did earlier after castration in pubertal and adult rats. Thus, after a first period characterized as independent of the gonads, it appears that the long-term preservation of the hypothalamic LHRH content in immature rats is determined by gonadal factors, as previously suggested by *Barnea* et al. [3]. Since early depletion of hypothalamic LHRH stores after castration of adult 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 work are to be considered. For instance, the mean serum 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 LHRH content measured in sham-anesthetized 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].

In conclusion, the measurement of tissue LHRH content indicates that the reaction of the hypothalamus to castration depends on the animal's stage of maturation. The study of LHRH release estimated either through its direct measurement or indirectly by analyzing the pulsatility of LH circulating levels could certainly help to a more complete understanding of the maturation of the hypothalamus during puberty.

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