

Orchiectomy in Young Rats Results in Differential Regulation of Follicle-Stimulating Hormone and Luteinizing Hormone Content

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While it is generally accepted that GnRH stimulates release of pituitary gonadotropins, it is not clear what regulates synthesis. The orchiectomized immature rat, with sustained high plasma levels of LH and FSH, provides an opportunity to study how gonadotropin biosynthesis responds to loss of the gonad.

We have measured plasma and pituitary LH and FSH in castrate and sham operated rats after orchiectomy at 15, 30, 45, and 60 days of age. Plasma FSH and LH concentrations by RIA were markedly elevated in castrates within one to three days after castration, and they remained elevated in all groups. By contrast, pituitary content measurements revealed differences between the two gonadotropins: while LH content in castrates consistently exceeded that in controls, FSH content in castrates was lower. Pituitary LH excess was evident by seven to ten days after castration. The pituitary FSH deficit in younger animals was similarly apparent by seven to ten days. In the older groups, however, FSH content decreased as early as three days, but returned toward normal by 21 days.

In orchiectomized young rats, pituitary LH is elevated, but FSH content is depressed. This discrepancy is delayed, but more marked, in younger rats. In view of sustained high plasma levels after castration, our findings imply differential regulation of synthesis, processing, or storage of the two gonadotropins.

Key words: LH, FSH, synthesis, regulation.

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Proper development and function of the CNS-pituitary-testicular axis is dependent on intricate feedback relationships between products of the

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hypothalamus, pituitary, and gonad. The main testicular functions, spermatogenesis and steroid hormone production, are known to be regulated at least in part by the pituitary gonadotropic glycopeptides, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Eik-nes, 1975; Means, 1975).

Removal of the gonadal sex steroids is followed by release of hypothalamic gonadotropin releasing hormone (GnRH), which in turn stimulates secretion of both pituitary gonadotropins (Piacsek and Meites, 1966; Redding et al, 1972; Eskay et al, 1977; Badger et al, 1978). Other testicular products may be involved in feedback, but this remains controversial (Sherins et al, 1982). Maturity of the hypothalamic-pituitary-gonadal axis may also modify the response to sex steroid deprivation. In the rhesus monkey, neonatal orchiectomy results in an initial elevation of plasma FSH and LH, but this increase is not sustained (Plant, 1980). By contrast, the neonatal rat demonstrates prolonged hypersecretion of FSH and LH following orchiectomy (Swerdlow et al, 1971). The rat, therefore, provides an opportunity to assess how FSH and LH biosynthetic responses to loss of the gonad contribute to the high plasma levels seen in this species throughout development.

Methods

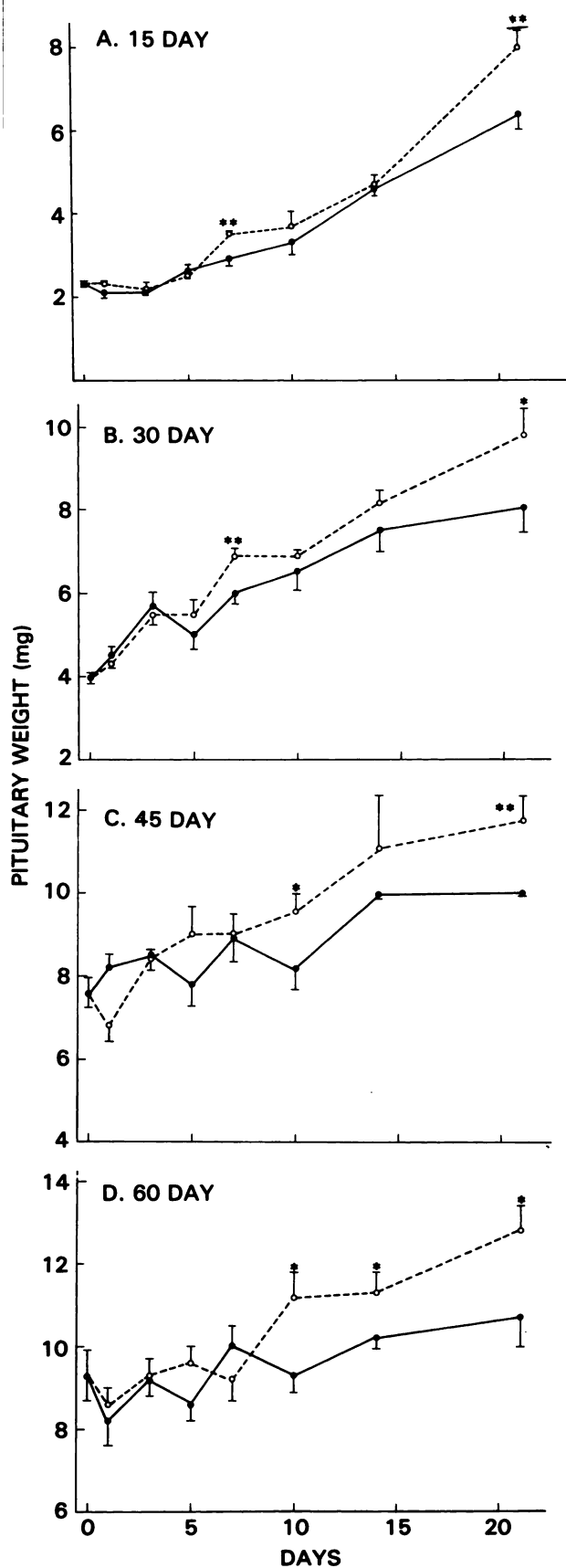
Animals

Male Holtzmann rats were obtained at 15, 30, 45, and 60 days of age. Fifteen-day-old pups were kept in groups

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of 16 with two lactating mothers until weaned on day 22. All animals 22 days or older received Purina Rat Chow and tap water *ad libitum*. They were housed at six to 12 rats per cage (fewer with increasing size) in a 23 C room lit from 7:00 A.M. to 7:00 P.M.

Experimental Design

At the start of the experiment (day 0), 48 rats of each age group (15, 30, 45, or 60 days) were castrated by the trans-scrotal route under light ether anesthesia, and 48 littermates were subjected to a sham operation. Operations were performed between 1:00 P.M. and 5:00 P.M.

Six intact animals were sacrificed on day 0. On each of days 1, 3, 5, 7, 10, 14, and 21, six castrated and six sham controls were sacrificed by decapitation between 8:00 A.M. and 11:00 A.M. Blood was collected in heparinized tubes, centrifuged, and the plasma separated and temporarily kept on ice. Whole pituitary glands were removed, weighed, and each placed in a separate tube containing 1 ml ice-cold radioimmunoassay (RIA) buffer (PBS, pH 7.6, 0.85% NaCl, 2.5% normal rabbit serum) and sonicated at 40 watt seconds for 5 seconds. Aliquots of each plasma and pituitary sample were stored at -20°C for hormone determination.

Measurement of FSH and LH

Plasma and pituitary gonadotropin concentrations were measured by double-antibody RIA using FSH-RP-1 and LH-RP-1 standards and antisera provided by the NIADDK National Hormone and Pituitary Program. I-125-iodoFSH and I-125-iodoLH were prepared using lactoperoxidase.

RIA was performed as previously described (Krueger et al, 1974). Plasma or pituitary gonadotropin levels were measured in the same assay to avoid between-assay variation. The limits of detection were 16.3 ng/ml for FSH and 1.1 ng/ml for LH. Within-assay variation was 10% at a dose of 100 ng/tube for FSH, and 13% at a dose of 20 ng/tube for LH.

Statistical Analysis

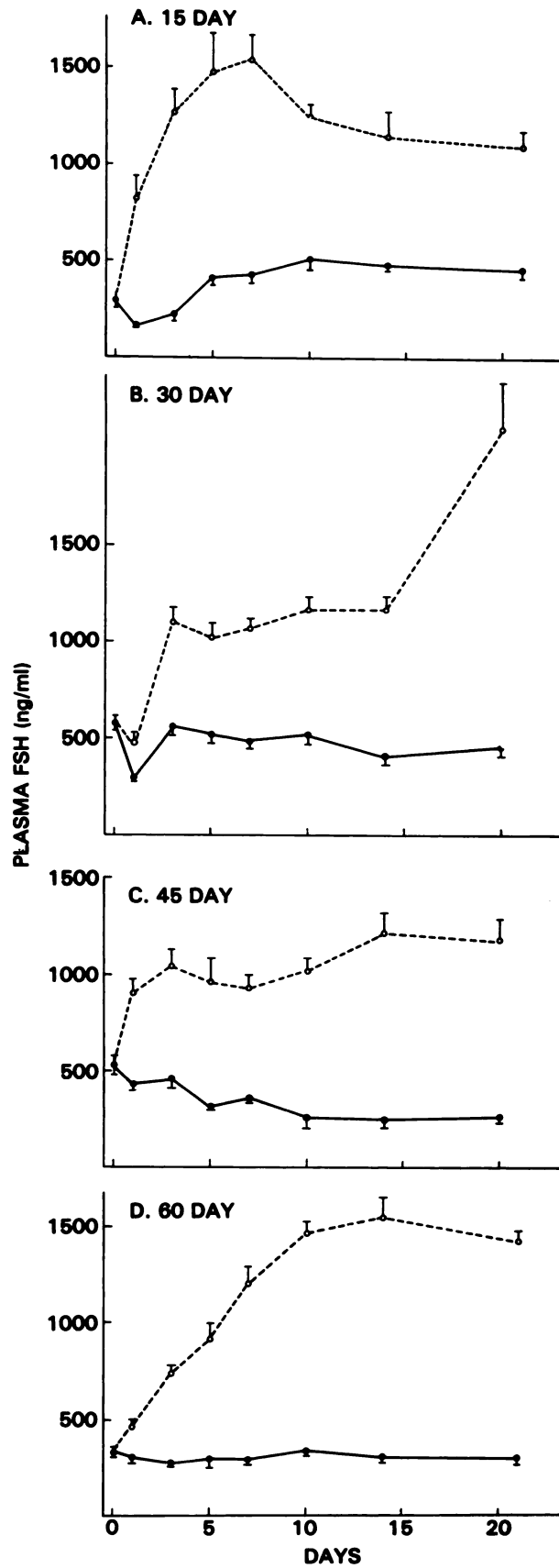
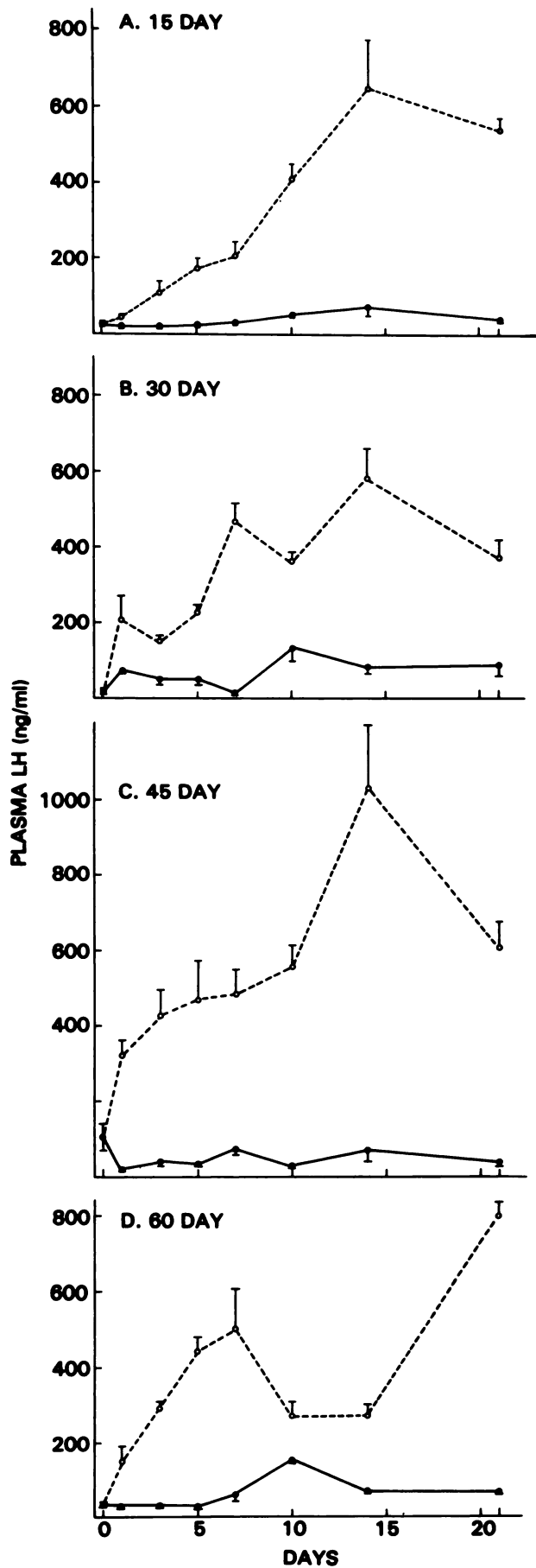
After logarithmic transformation of the data to achieve uniformity of variance, analysis of variance (ANOVA) was performed using the Statistical Analysis System. Results are expressed as the mean \pm SEM. Significance of difference between the control and castrate groups for each time point was determined by Student's *t* test (two-tailed).

Results

Pituitary Weights

Figure 1 shows the progressive rise in pituitary weight for rats castrated or sham-operated at 15,

Fig. 1. Pituitary weight (mg) in rats castrated (O—O) or sham operated (●—●) at 15(A), 30(B), 45(C), or 60(D) days of age, from 0 to 21 days after operation. Mean \pm SEM. * = $P < 0.05$. ** = $P < 0.01$.



Figs. 2 and 3. Plasma LH (Fig. 2, left) and FSH (Fig. 3, above) concentration (ng/ml) in rats castrated (O—O) or sham operated (●—●) at 15(A), 30(B), 45(C), or 60(D) days of age, from 0 to 21 days after operation. Mean \pm SEM.

30, 45, or 60 days of age. At 21 days after operation, all castrates showed increased growth relative to controls ($P < 0.05$). No differences between castrate and sham were seen earlier than seven days after operation.

Plasma Gonadotropins

Plasma LH (Fig. 2) and FSH (Fig. 3) rose rapidly in all castrates, and remained markedly elevated. In most groups, the increase was apparent within 24 hours; it was significant in all groups three days after castration ($P < 0.05$). While FSH rose to three to six times that of the age-matched control levels, plasma LH increased seven- to 13-fold.

Pituitary Gonadotropins

Figures 4 and 5 show pituitary contents of LH and FSH, respectively, in rats castrated or sham operated at 15, 30, 45, or 60 days of age. In contrast to the rapid rise in plasma LH, the increase in pituitary LH was evident no earlier than seven days after castration in any group. After that time point the pituitary LH content in castrates exceeded controls (Fig. 4) while pituitary FSH content in castrates (Fig. 5) was generally lower than in controls ($P < 0.05$).

The 15 and the 30 day groups demonstrate reduced pituitary FSH in the castrate compared to controls ten and five days, respectively, after operation. In the 45- and 60-day groups, however, the castrates showed lower FSH content as early as three days, which then returned toward normal at 14 and 21 days.

ANOVA yielded high levels of significance ($P < 0.0001$) for the effect of castration upon pituitary weight and plasma and pituitary gonadotropins. The maximal increase or decrease in each hormonal parameter compared to the appropriate sham control was comparable in magnitude for each age group. However, the time course of castration effects was age-dependent ($P < 0.005$). The effect of castration upon all measured parameters was confirmed in a separate duplicate experiment (data not shown).

Discussion

In the rat, brisk and sustained release of both FSH and LH follows removal of the negative feedback of the gonad. We have shown for the first

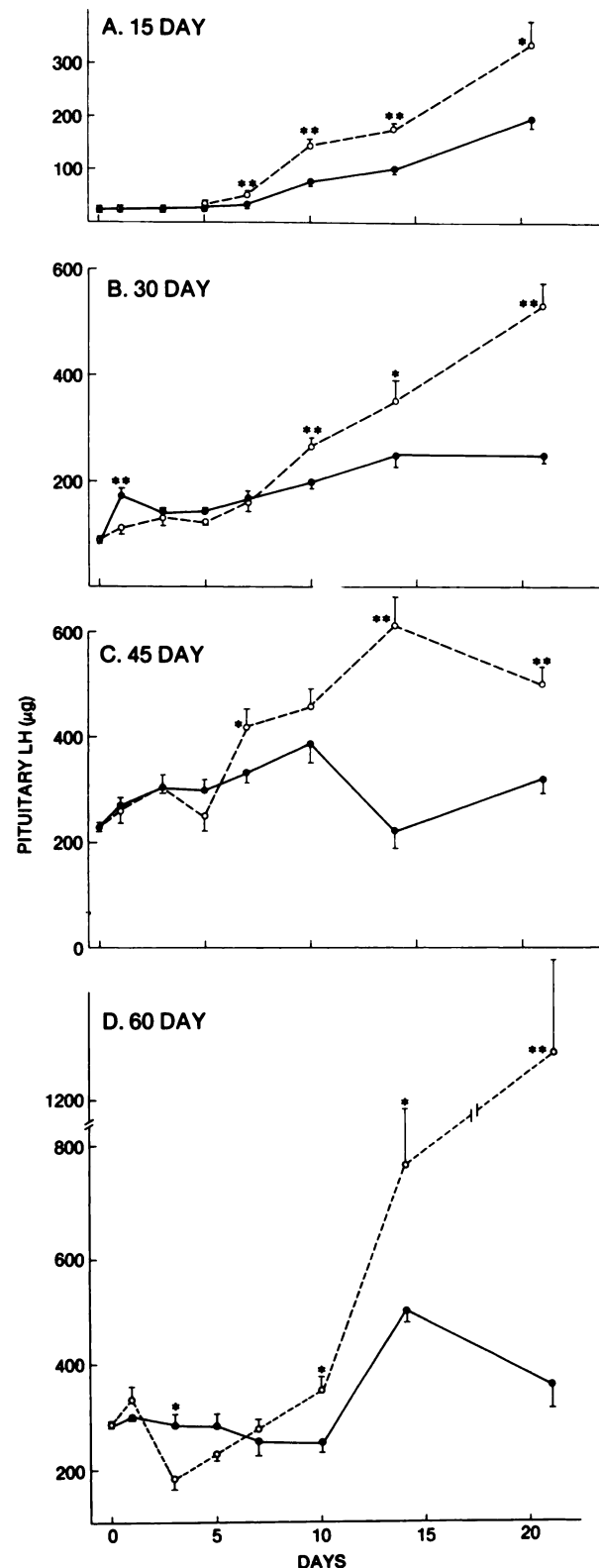


Fig. 4. Pituitary LH content (μg per pituitary) in rats castrated ($\text{O} \text{---} \text{O}$) or sham operated ($\bullet \text{---} \bullet$) at 15(A), 30(B), 45(C), or 60(D) days of age, from 0 to 21 days after operation. Mean \pm SEM. * = $P < 0.05$. ** = $P < 0.01$.

time, however, a disparity between levels of the two gonadotropins in pituitary tissue of young castrated rats relative to intact controls. Since pituitary content reflects the net result of synthesis, processing, storage, and release, this disparity implies differential regulation at one or more of these stages for the two hormones.

A possible explanation for our findings might be the effect on the pituitary of high postcastration levels of GnRH. While it is known that GnRH release leads to secretion of preformed FSH and LH (Samli and Geschwind, 1967; Redding et al, 1972; Ketelslegers et al, 1978), it is not yet resolved whether GnRH affects the proximal events of synthesis, processing, and storage. Several groups have found that GnRH does stimulate glycosylation as well as release (Liu et al, 1976; Menon et al, 1977; Azhar et al, 1978; Khar et al, 1978), but these experimenters disagree as to the effects on peptide synthesis. Glycosylation or combination of subunits, as required for full biological activity of glycoprotein hormones (Reichert and Ward, 1974; Moyle et al, 1975; Sairam 1980, Weintraub et al, 1983), might be slower or less complete for FSH. It is also possible that FSH may be released sooner after its manufacture, without accumulating in the gland.

There is recent evidence for differences in bioactivity among gonadotropins secreted under different hormonal conditions (Mukhopadhy et al, 1979; Solano et al, 1980). We did not measure gonadotropin bioactivity in our present developmental study, as this was not the goal of the experiment; however, this area would be of interest for future investigation.

The discordance we have seen in gonadotropin content may be due to differences in the numbers of cells making LH and FSH. It appears from immunocytochemistry (Childs et al, 1980) that FSH and LH are usually present in the same cells, although a small percentage of pituicytes show only one hormone. Castration may provoke preferential proliferation or recruitment of LH-producing cells. Alternatively, LH- and FSH-producing cells may be differentially sensitive to such effectors as extragonadal sex steroids (Wakabayashi and Tamaoki, 1967; Chowdhury and Steinberger, 1976; Drouin and Labrie, 1976) or GnRH. Pituicyte GnRH receptor number is known to rise after castration (Clayton and Catt, 1981) or GnRH treatment (Pieper et al, 1982) in adult male rats, but it

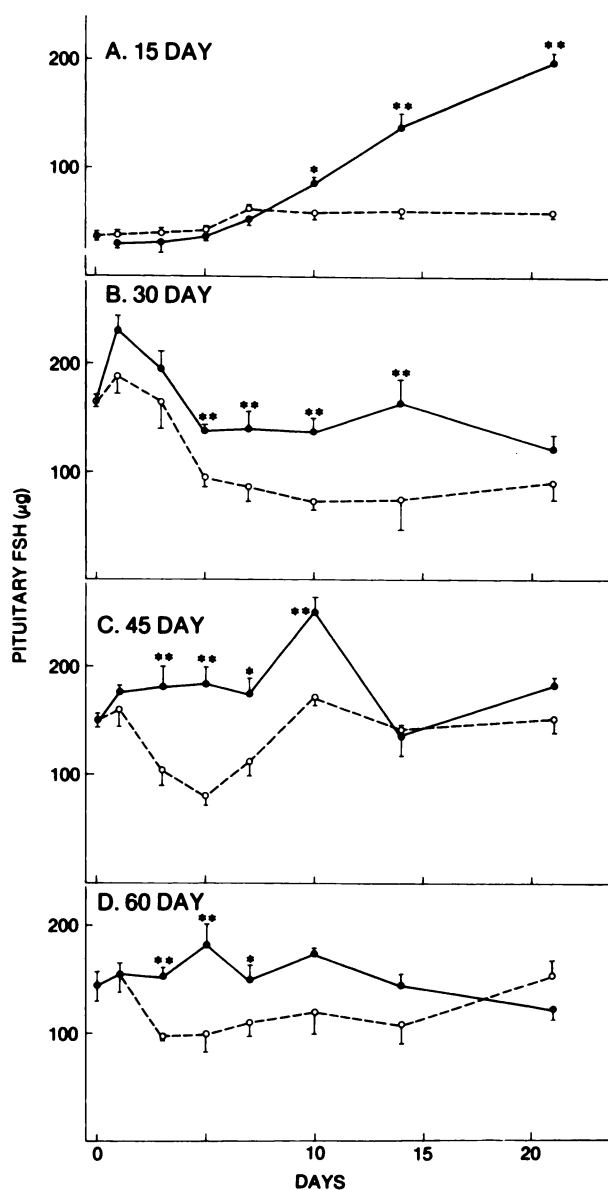


Fig. 5. Pituitary FSH content (μg per pituitary) in rats castrated ($\text{O} \text{---} \text{O}$) or sham operated ($\bullet \text{---} \bullet$) at 15(A), 30(B), 45(C), or 60(D) days of age, from 0 to 21 days after operation. Mean \pm SEM. * = $P < 0.05$. ** = $P < 0.01$.

is not yet known if LH- and FSH-producing cells differ in this response.

Age is also a factor in the differential responses of LH and FSH. Although the shortfall of pituitary FSH after castration is more marked in juvenile rats than in adults, it occurs later. In the rat, the hypothalamic-pituitary-testicular axis is functional early in life (Swerdlow et al, 1971; Goldman et al, 1971; Ojeda and Ramirez, 1972; Negro-Vilar et al,

1973; Gupta et al, 1975; Ketelslegers et al, 1978). Gonadotropes constitute a greater percentage of pituicytes in seven- to 15-day-old male rats than in adults (Childs et al, 1981). Pituitary GnRH receptors reach a maximum at 30 days of age (Dalkin et al, 1981). Thus, the relatively numerous GnRH-sensitive cells may represent a greater gonadotropin reserve in the juvenile rat.

An early fall and late recovery of pituitary FSH is suggested by the work of Caraty et al (1981a) in rats castrated at 120 days of age and by Badger et al (1978) in rats castrated at 60 days. However, the scatter in those data prevented ascribing significance to the trends. Our data differ from those of Caraty et al (1981b), who reported increased pituitary FSH in 17-day-old rats one week after castration.

Recent studies using cell-free translation of mRNA from adult rat pituitaries indicates that formation of gonadotropin subunit precursors is greater in castrates (Godine et al, 1980), and that translation of LH- β mRNA exceeds that of FSH- β (Counis et al, 1982). Preliminary data from our laboratory on intracellular processing show increased amounts of glycosylated LH subunits after castration (Vogel et al, 1983). Immunohistochemical and pulse-chase experiments now in progress should allow us to distinguish between translation, processing, and storage as explanations for the results reported here.

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Opioid Peptides in Periphery

The Symposium on Opioid Peptides in Periphery will be held in Rome, Italy on May 23-25, 1984. The Symposium is sponsored by the National Research Council-CNR, Italy, the Italian Society of Endocrinology, and the Italian Society of Andrology. It will consist of main lectures, free communication, poster sessions, and round table discussions. The Symposium is devoted to the biochemical, physiological, and clinical aspects of the opioid peptides at peripheral levels. It will be interdisciplinary, involving opioid peptides and: andrology, circulation, endocrinology, gastroenterology, immunology, and obstetrics and gynecology. The stress will be placed on methods of detection and visualization, clinical aspects, and physiological role.

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