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A reinvestigation of hypothalamic-pituitary testicular interactions : simultaneous changes in tissue and plasma levels of gonadotrophins, prolactin, testosterone and hypothalamic LH-RH after bilateral orchidectomy and cryptorchidism

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Summary. Plasma concentrations of LH, FSH, prolactin and testosterone, pituitary concentrations of gonadotrophins and prolactin, and hypothalamic LH-RH were measured in normal, sexually mature male rats at regular intervals from 1 to 30 days after castration or cryptorchidism. Bilateral castration resulted in a marked decrease in testosterone levels 24 h after surgery. On the contrary, plasma testosterone was increased (at days 4 and 8) or unaffected by cryptorchidism when compared to intact controls. From day 1, castrated rats showed a rapid, marked increase in plasma LH and FSH (7 and 5 times higher at day 30 for LH and FSH, respectively) and a delayed, progressive increase in pituitary gonadotrophins (significant at day 8 and day 30 for LH and FSH, respectively). The cryptorchid animals showed similar but slower and less marked changes in plasma and pituitary FSH and LH levels. Unlike plasma prolactin levels, which were lowered at day 8 or days 4, 8 and 30, respectively, after castration or cryptorchidism, no change was observed in pituitary prolactin content.

After castration, hypothalamic LH-RH content was significantly lower at day 4 and gradually decreased until day 30. On the other hand, no changes were observed after cryptorchidism.

The present observations indicate that factors other than testosterone, associated with the presence of an active seminiferous epithelium, are involved in the regulation of gonadotrophin secretion. These factors do not seem to change the hypothalamic LH-RH content.

Introduction.

The dual function of the testis is well recognized, but the complex hormonal and neurohormonal processes involved in regulating testicular functions are still only partly understood. Surgical induction of cryptorchidism in the adult rat reduces the weights of the testis and the accessory glands (Amatayakul *et al.*, 1971 ; Swerdloff *et al.*, 1971).

Moreover, plasma gonadotrophin levels are increased shortly after surgery (Amatayakul *et al.*, 1971 ; Gomes and Jain, 1976), while pituitary concentrations

change after a longer time-lag (Steinberger and Duckett, 1966 ; Amatayakul *et al.*, 1971). There is little agreement on the effect of experimental cryptorchidism on plasma testosterone levels : Amatayakul *et al.* (1971) and Kerr, Rich and de Kretser (1979) found these levels depressed by about 50 p. 100 from day 7 to day 8 after surgery, while Gomes and Jain (1976) and Hopkinson *et al.* (1979) found the levels were unchanged for 30 days. Furthermore, at 30 days, the seminal vesicle weights were not lower (Amatayakul *et al.*, 1971 ; Gomes and Jain, 1976). As the damage to the seminiferous tubule, induced by cryptorchidism, seems to be established at around day 14 (Clegg, 1963 ; Amatayakul *et al.*, 1971), it was thought interesting to reexamine the effects of cryptorchidism on some of the hypothalamo-hypophyseal functions by comparing these effects to those of castration, paying special attention to the first two post-operative weeks. Thus, plasma levels of gonadotrophins (FSH and LH), prolactin (PRL) and testosterone and pituitary levels of FSH, LH and PRL, as well as hypothalamic levels of luteinizing hormone-releasing hormone (LH-RH), were measured at intervals after surgery.

Material and methods.

Adult male Wistar rats (INRA strain 03), 120 days old and weighing 400 to 450 g, were kept in a controlled light environment (14 hrs light-10 hrs dark) throughout this study.

Nembutal anaesthesia (5 mg/100 g body weight) was used for all surgical procedures.

A total of 144 rats was randomly divided into three groups :

1) Cryptorchid group : the testes and their appendages were gently lifted out of the scrotal sac through a midline abdominal incision and placed in the abdominal cavity after severing the gubernaculum. The inguinal canal and the scrotal sac were then completely obliterated with silk purse sutures. Repeated examination showed that the testes did not return to the scrotum and were not ischaemic.

2) Castrated group : ablation of the testes and the epididymides was performed via the scrotal route.

3) Sham-operated group : the animals were submitted to an operation similar to that performed in the cryptorchid group, except that the gubernaculum was not severed and the scrotal sac and inguinal canal were left open. On repeated occasions after surgery, the testes were observed in the scrotal position.

Eight rats in each group were slaughtered by rapid decapitation between 09.00 hr and 10.30 hrs, 1, 2, 4, 8, 15 and 30 days after surgery. The pituitary, hypothalamus and blood were collected from each animal. The anterior pituitaries from each group of 8 animals were pooled, homogenized in deionized water, lyophilized, extracted with saline and frozen until assay. Each hypothalamus, including the pituitary stalk, was collected immediately after decapitation, homogenized in cold 0.1 N HCl, sonicated (15" at maximal power using a MSE 150 W ultrasonic disintegrator) and frozen. The limits of the hypothalamic samples were defined as the optic chiasma rostrally, the hypothalamic fissures laterally and the mamillary bodies posteriorly. Each hypothalamic section was approximately 2 to 3 mm in depth. Blood samples were collected

under heparin and after centrifugation at 4 °C, the plasmas were stored at — 20 °C until assayed.

LH-RH content was estimated by a specific radioimmunoassay (Caraty *et al.*, 1980). The intra-assay coefficient of variation was 8 p. 100 and the detection limit was 0.4 pg per tube.

Pituitaries and plasma were analyzed for FSH content using the NIAMDD radioimmunoassay kit. The results are expressed in terms of NIAMDD rat FSH-RP 1.

Pituitary and plasma LH contents were measured using a specific double antibody radioimmunoassay method (Viguier-Martinez and Hochereau-de-Reviers, 1977). One unit of the purified hormone used for radioiodination and standard (LHS × 1-1) * was equivalent to 1.58 unit of NIH-LH S 11.

Plasma and pituitary prolactin levels were measured using a specific raioimmunoassay method (Martinat *et al.*, 1979). The intra-assay coefficient of variation was 10 p. 100 and the detection limit was 0.32 ng/ml plasma. The potency of the standard (PRL-INRA) was about twice that of the NIAMDD-rat PRL-RP 1. Plasma testosterone was measured after solvent extraction (3 ml ethyl ether per 0.1 ml plasma) using a radioimmunoassay method (Picaper, in preparation). Antibody was obtained in the rabbit by injection of testosterone 3 (0-carboxymethyl)-oxime conjugated to bovine serum albumin. The cross-reactions of the antiserum were : 49 p. 100 with 5α-dihydrotesterone ; 1.9 p. 100 with Δ_4-androstenedione ; < 1 p. 100 with progesterone, œstradiol, cortisol and 3α and 3β-androstanediol. The intra-assay coefficient of variations was 6 p. 100 and the detection limit 50 pg/ml.

All the samples of each hormone were processed in duplicate (plasma) or quadriplicate at five doses (pituitaries) in a single assay. The hormone concentrations of plasma samples were determined using logit-log transformation. Only those two of the five doses of pituitary extracts nearest the $B/B_o = 50$ p. 100 were used for a 4-point assay (Emmens, 1948). The results were expressed in terms of standard per mg lyophilized powder. A comparison of means between the experimental and the control groups was conducted using the t-test for independent means (Snedecor, 1956).

Results.

Plasma and pituitary levels of LH (figs. 1*a*-2*b*). — After gonadectomy, a rapid and marked increase in plasma LH occurred between day 1 ($P < 0.01$) and day 15 ($P < 0.001$). These elevated levels then remained roughly constant between day 15 and day 30 (about 7 times the levels of the sham-operated animals). Twenty-four hours after surgery, pituitary LH concentration was significantly ($P < 0.05$) lower, but a progressive increase in pituitary concentration was noticeable from day 8 ($P < 0.05$) until day 30 ($P < 0.001$). At that time, the pituitary LH concentration of the castrated group was three fold greater than that of the sham-operated animals.

In cryptorchid animals, plasma LH levels rose between day 8 and day 15 ; the values then remained approximately constant (from day 15 to day 30) at about twice those of the levels observed in sham-operated rats. Pituitary LH content was only significantly ($P < 0.001$) greater than in sham-operated controls at day 30.

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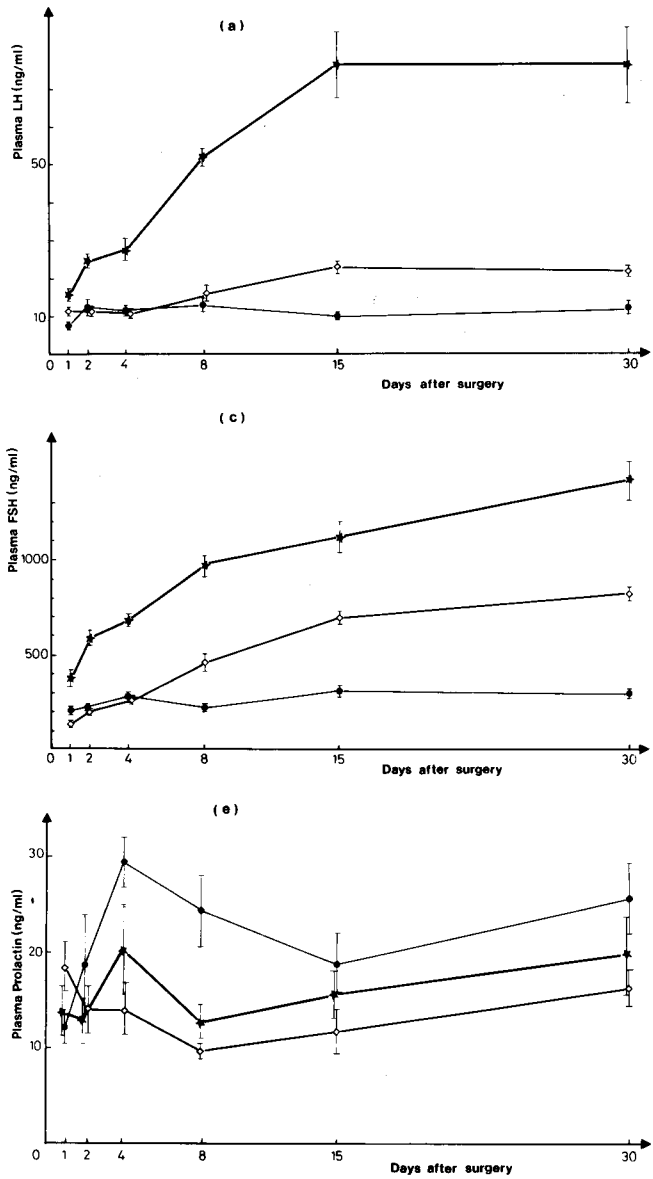

FIG. 1. — *Plasma levels of LH, FSH or PRL in adult male rats after surgical cryptorchidism* (◇—◇), *castration* (★—★) *or sham operation* (●—●). Standards are CNRS-LH Sx 1-1 ; NIAMDD FSH RP1 ; INRA-PRL (equivalent to 2 times the NIAMDD PRL-RP1). Error bars represent SEM.

Plasma and pituitary levels of FSH (figs. 1*c*-2*d*). — After castration, plasma FSH levels rose between days 1 and 30. Plasma FSH concentrations were always significantly greater ($P < 0.01$) in castrated than in sham-operated animals. In the cryptor-

chid rats, plasma FSH levels were depressed at day 1 but increased from day 8 onwards ($P < 0.001$). A plateau was reached about 15 days after surgery. Plasma FSH levels in castrated and cryptorchid animals were about 5 or 2.5-fold greater, respectively, than

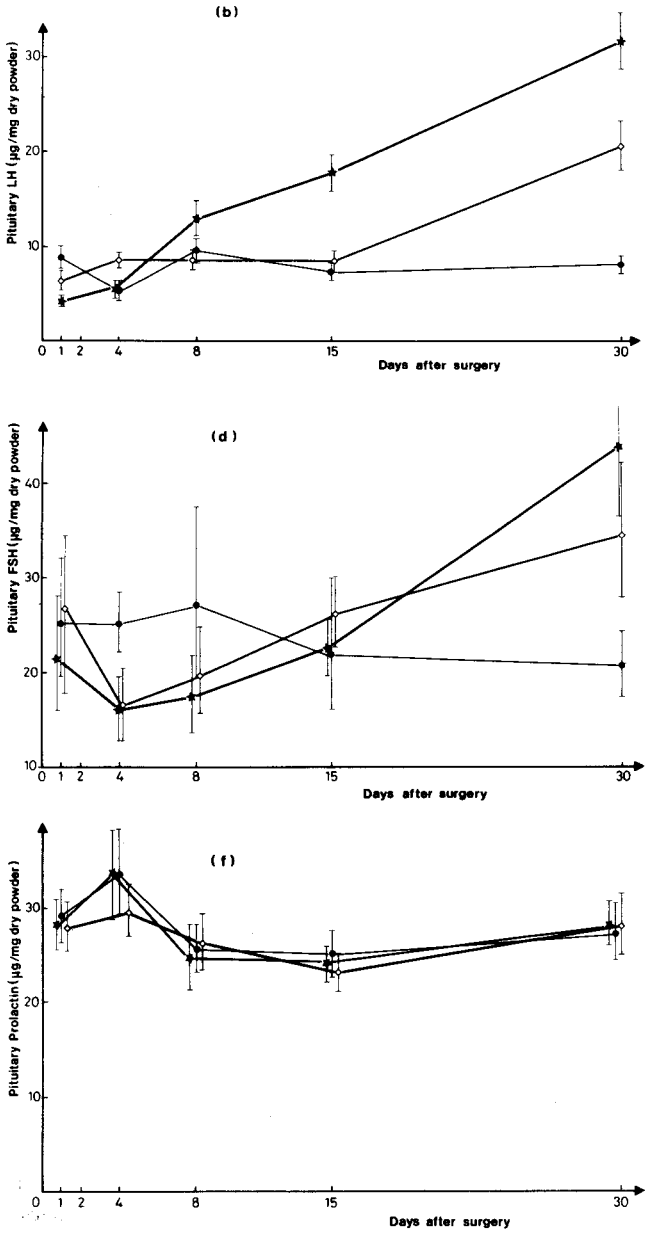

FIG. 2. — *Pituitary LH, FSH or PRL content in adult male rats after surgical cryptorchidism* (◇—◇), *castration* (★—★) *or sham operation* (●—●). Error bars represent fiducial limits (P = 0.95).

control values at day 30. Pituitary FSH concentrations were lower at day 4 ($P < 0.05$) in castrated and cryptorchid animals and did not increase before day 30. At that time, the values were 2 or 1.7-fold greater than the control levels for the castrated and cryptorchid groups, respectively.

Plasma and pituitary levels of prolactin (figs. 1e-2f). — Plasma prolactin levels were depressed 8 days after castration ($P < 0.01$) and 4 and 8 days ($P < 0.001$) and 30 days ($P < 0.05$) after cryptorchidism when compared with sham-operated animals. Furthermore, from day 4 to 30 cryptorchid rats had significantly lower plasma prolactin levels than castrated animals ($P < 0.05$).

No significant variation was observed in pituitary prolactin concentration at any time after castration or cryptorchidism.

Plasma level of testosterone (fig. 3). — Twenty-four hours after castration, plasma testosterone levels were significantly depressed ($P < 0.001$). These low levels remained unchanged between days 1 and 8, then increased significantly at day 15 and day 30 ($P < 0.01$ and $P < 0.001$, respectively, when compared with day-1 levels). Cryptorchidism induced a slight increase in plasma testosterone concentration during the first week after surgery (day 4 : $P < 0.05$; day 8 : $P < 0.01$). Thereafter, the plasma levels were indistinguishable from the control values.

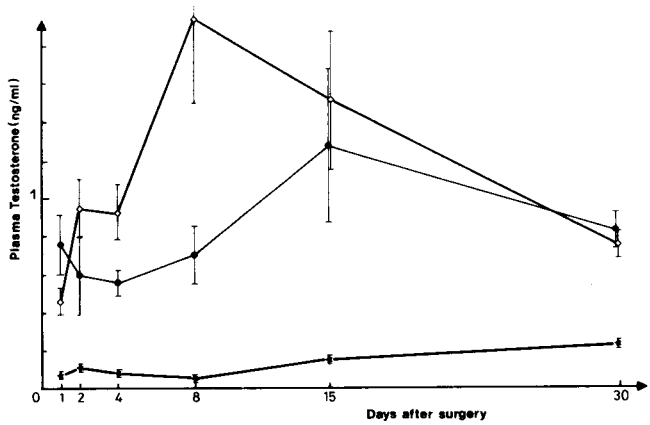

FIG. 3. — *Testosterone plasma levels in adult male rats after surgical cryptorchidism* (◇—◇), *castration* (★—★) *or sham operation* (●—●). Error bars represent SEM.

Hypothalamic LH-RH content (fig. 4). — The hypothalamic LH-RH content decreased significantly ($P < 0.01$) at 4 days after castration and continued to fall until day 30. In contrast, no change was observed at any time after cryptorchidism.

Discussion.

The present results suggest that surgical cryptorchidism induces an increase in plasma testosterone during the first week after surgery. The increase at day 4 has not been found by some authors (Gupta *et al.*, 1975 ; Gomes and Jain, 1976), and its signifi-

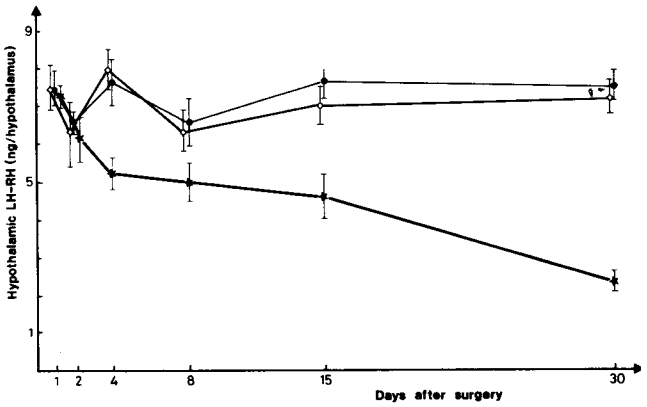

FIG. 4. — *Hypothalamic LH-RH in adult male rats after cryptorchidism* (◇—◇), *castration* (★—★) *or sham operation* (•—•). Error bars represent SEM.

cance ($P < 0.05$) may be questioned, but there is good agreement with other investigators (Gomes and Jain, 1976 ; Hall and Gomes, 1975) concerning the reported rise at day 8 ($P < 0.01$). The latter authors, however, did not report the day 8 rise as significant.

In addition, it must be pointed out that Gomes and Jain (1976) have shown a significant increase of plasma testosterone in unilateral cryptorchid animals at day 4.

It is interesting that Gomes and Jain (1976) observed an augmentation of seminal vesicle weight in the cryptorchid rat 8 days after surgery. Although the increase in plasma testosterone levels observed around day 8 is difficult to explain it may be hypothesized that the higher FSH levels at day 8 augmented the LH-induced testosterone response as shown previously by Johnson and Ewing (1971) and Chen, Payne and Kelch (1976). There is general agreement on the similarity of testosterone values between cryptorchid and intact controls in the ensuing period : around day 15 (Gomes and Jain, 1976 ; Hopkinson *et al.*, 1979 ; above results) and around day 30 (Hopkinson *et al.*, 1979 ; de Kretser, Sharpe and Swanston, 1979 ; Kerr, Rich and de Kretser, 1979 ; above results).

The present results agree in general with previous reports (castration : e. g. Amatayakul *et al.*, 1971 ; Badger *et al.*, 1978 ; cryptorchidism : Amatayakul *et al.*, 1971 ; Gomes and Jain, 1976 ; Hopkinson *et al.*, 1979) on the effects of castration or cryptorchidism on plasma and pituitary gonadotrophin levels in adult rats. The plasma and pituitary values of cryptorchid animals, for each of these hormones, lie somewhere between those of intact and castrated animals. However, a precise comparison reveals the following differences between the present and other results. In cryptochid animals, Amatayakul *et al.* (1971) indicated that plasma LH levels rise at day 2 after surgery, before those of FSH. On the contrary, our results, like those of Gomes and Jain (1976) and Hopkinson *et al.* (1979), show that plasma FSH levels were affected before those, of LH. However, the significant rise in plasma FSH levels takes place at day 2 or 4 according to those authors, instead of at day 8 (present results). Stress or surgical pro-

cedures may account for these differences, but the present values show a significant rise at day 4 (when compared to day-1 values for cryptorchid animals) instead of the day-4 values for sham-operated controls.

Furthermore, at 30 days after cryptorchidism and castration, an increase of both plasma and pituitary FSH and LH occurs which argues in favour of a rise in the synthesis of both gonadotrophins. The post-castration and post-cryptorchidism changes in pituitary FSH and LH observed in this study agree with earlier findings (Steinberger and Duckett, 1966 ; Amatayakul *et al.*, 1971).

Plasma prolactin levels in intact rats were found to be intermediate between those reported by Shin *et al.* (1974) or Sharr *et al.* (1975) (50-100 ng PRL-RP 1) and Ojeda, Jameson and McCann (1976) (10 ng/ml). Decapitation was chosen to avoid any stress response (Ojeda, Jameson and McCann, 1976), and the different levels observed by these authors may result from the younger rats used in the experiment. Nevertheless, the lowest plasma prolactin values at day 1 and day 2, when compared to values between day 4 to day 30 for sham-operated animals, may be attributed to the anaesthesia. Effectively, Borrell, Piva and Martini (1978) reported a decrease of plasma prolactin levels 48 h after intraperitoneal administration of sodium pentobarbital to adult rats.

As shown previously (Shin *et al.*, 1974 ; Shaar *et al.*, 1975), plasma prolactin levels were decreased slightly after castration. Cryptorchidism also depressed plasma prolactin levels, although this phenomenon was not observed by Ojeda, Jameson and McCann (1976). This, too, may be related to the age of the animals. Cryptorchidism (Ojeda, Jameson and McCann, 1976 ; Martinat, unpublished data) or castration (Martinat *et al.*, 1979) of prepuberal rats has no effect on plasma prolactin levels. It has been shown that estradiol (Kalra *et al.*, 1973 ; Shin, 1979), testosterone (Kalra *et al.*, 1973 ; Shin *et al.*, 1974) and aromatizable androgens, but not non-aromatizable androgens (Nolin *et al.*, 1977 ; de La Heras and Negro-Vilar, 1979), are able to raise plasma prolactin levels. It is possible, therefore, that estradiol secretion may have been altered in the cryptorchid animals, as proposed by Hall and Gomes (1975). However, this does not explain why, in the present experiment, prolactin levels were more depressed in cryptorchid than in castrated animals.

The hypothalamic LH-RH content found in the adult rats in our experiment is very similar to that reported by Chen, Geneau and Meites (1977), Payne *et al.* (1977), Badger *et al.* (1978), Kalra and Kalra (1978) (6 to 8 ng) but somewhat different than that published by other authors (Chiappa and Fink, 1977 : 12 ng ; Shin and Howitt, 1974, 1975 : 15 to 20 ng ; Root *et al.*, 1975 : 2 ng). The very short-term effect of castration, namely the augmentation of LH-RH hypothalamic content at day 1 or day 2 (Shin and Howitt, 1975), was not observed here. This is in agreement with the reports of Shin and Howitt (1976) and Chen *et al.* (1977). On the other hand, the decrease in hypothalamic LH-RH content known to occur around day 7 (Shin and Howitt, 1975 ; Root *et al.*, 1975 ; Chen *et al.*, 1977 ; Badger *et al.*, 1978), was observed from day 4 onwards. This 50 p. 100 decrease in hypothalamic content is associated with an increased LH-RH concentration in the hypophyseal portal blood and an increase in the synthesis of the neurohormone by the hypothalamus (Eskay, Mical and Porter, 1977 ; Moguilevsky, Enero and Szwaracfarb, 1974 ; Moguilevsky *et al.*, 1975 ; Kochman, Kochman and Domanski, 1977). The decreased LH-RH content appears to be related to the fall in circulating androgens because testosterone treatment of orchidectomized rats restored their level

to that of the intact controls (Shin and Howitt, 1975 ; Chen *et al.*, 1977 ; Kalra and Kalra, 1978). Interestingly, cryptorchidism does not affect the hypothalamic LH-RH content for up to 30 days, further supporting the preceding data. This fact, coupled with the absence of any modification of plasma testosterone levels, strongly suggests that the LH secretion effects observed in the cryptorchid are largely due to modifications at the pituitary level.

Since the increase in plasma LH and FSH in response to LH-RH was greater in cryptorchid than in intact animals (Hopkinson *et al.*, 1979), this hypothesis appears justified. In addition, it has been shown that media from cultures of mature rat seminiferous tubules, ovine testicular lymph and ovine RTF, respectively, are able to suppress, in a dose-related manner, both the basal secretion and the LH-RH stimulated secretion of FSH and LH by cultured rat pituitary cells (Eddie *et al.*, 1978, 1979 ; de Jong, Smith and Van der Molen, 1979).

The mechanism through which plasma LH and FSH levels are raised in the cryptorchid rat could be as follows : inhibin (for recent reviews see de Jong, 1979 ; Blanc, 1980), a factor from the germinal epithelium, secreted in greater quantities in the normal than in the abdominal testis, is able to decrease the sensitivity of the pituitary to LH-RH without affecting the hypothalamic LH-RH content.

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Résumé. Les concentrations plasmatiques de la LH, FSH, prolactine et testostérone, les contenus hypophysaires en gonadotropines et prolactine ainsi que le contenu hypothalamique en LH-RH ont été mesurés chez des rats mâles adultes à intervalles réguliers de 1 à 30 jours après la castration ou la cryptorchidie. La castration bilatérale entraîne, dès 24 h, post-opératoire une diminution brutale des taux de testostérone. Au contraire, chez les animaux cryptorchides, on observe une augmentation (à J4 et J8) ou pas de différence par rapport aux niveaux des animaux témoins. Chez les rats castrés, une rapide augmentation de la LH et de la FSH plasmatique intervient dès J1 et les niveaux de gonadotropines sont 7 et 5 fois plus élevés que ceux des contrôles à J30 respectivement pour la LH et la FSH. Chez les animaux cryptorchides, les changements observés dans les teneurs plasmatiques et hypophysaires en LH et en FSH sont comparables à ceux observés après castration mais interviennent plus tardivement et sont moins marqués.

Après castration ou cryptorchidie, aucun changement n'intervient dans les contenus pituitaires en prolactine. Par contre, les niveaux plasmatiques en prolactine sont significativement abaissés 8 jours post-castration et 4, 8 et 30 jours post-cryptorchidie. Au niveau hypothalamique, on observe après castration une diminution progressive des taux de LH-RH, significative dès J4 et qui s'accentue jusqu'à J30. Au contraire, aucun changement n'intervient après cryptorchidie.

Le présent résultat indique qu'un facteur différent de la testostérone, associé avec la présence d'un épithélium séminifère actif, est impliqué dans la régulation des gonadotropines sans provoquer de changement dans les contenus hypothalamiques en LH-RH.

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