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STRESS INDUCED CHANGES IN TESTIS FUNCTION

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Summary—The mechanism through which chronic stress inhibits the hypothalamic–pituitary–testicular axis has been investigated. Chronic restraint stress decreases testosterone secretion, an effect that is associated with a decrease in plasma gonadotropin levels. In chronically stressed rats there was a decrease in hypothalamic luteinizing hormone-releasing hormone (LHRH) content and the response on plasma gonadotropins to LHRH administration was enhanced. Thus the inhibitory effect of chronic stress on plasma LH and FSH levels seems not to be due to a reduction in pituitary responsiveness to LHRH, but rather to a modification in LHRH secretion. It has been suggested that β-endorphin might interfere with hypothalamic LHRH secretion during stress. Chronic immobilization did not modify hypothalamic β-endorphin, while an increase in pituitary β-endorphin secretion was observed. Since we cannot exclude that changes in β-endorphin secreted by the pituitary or other opioids may play some role in the stress-induced decrease in LHRH secretion, the effect of naltrexone administration on plasma gonadotropin was studied in chronically stressed rats. Naltrexone treatment did not modify the decrease in plasma concentrations of LH or FSH. These findings suggest that the inhibitory effect of restraint on the testicular axis is exerted at hypothalamic level by some mechanism other than opioids.

INTRODUCTION

It is well recognized that stress inhibits gonadal function in various animal species including humans (for review see [1]). Different stressful stimuli such as swimming, long-distance running, surgery, electric foot shocks and psychological stimuli have been shown to induce a decrease in plasma testosterone levels [2–8].

However, the precise mechanism through which stress decreases testosterone secretion is not well understood. It has been postulated that stress does not directly affect testicular testosterone secretion, but rather inhibits gonadotropin secretion acting at pituitary or hypothalamic level [5, 9]. Other authors have found that, although plasma concentrations of testosterone were decreased, there was no reduction in plasma LH levels after stress [10, 11].

Stress activates the hypothalamic–pituitary–adrenal axis and there is evidence suggesting a correlation between the degree of adrenal hyperfunction and testicular atrophy after chronic stress [12]. Since stress increases the secretion of glucocorticoids, endogenous opioids and corticotropin-releasing factor (CRF), the inhibition of the testicular axis caused by stress could be due to: (1) increased levels of glucocorticoids during chronic stress, which may interfere with testosterone [13, 14] or LH secretion [15, 16]; (2) increased release of endogenous opioids, which have been reported to inhibit luteinizing hormone-releasing hormone (LHRH) [17]; or (3) enhanced secretion of CRF, since this peptide is known to decrease LHRH release [18].

Therefore our studies were designed to examine: the effect of stress on the different hormones of the testicular axis and the possible role of glucocorticoids and endogenous opioids in the chronic stress-induced inhibition of the testicular axis.

EFFECT OF RESTRAINT STRESS ON THE TESTICULAR AXIS

Adult male Wistar rats weighing around 350 g were stressed by restraint in a small flexible wire mesh container. All rats were killed at 16.00 h after being stressed for 0, 20, 45, 90, 180 or 360 min; or after 6 h of daily immobilization (from 10.00 to 16.00 h) over 4 consecutive days.

As shown in Fig. 1, the testosterone response to stress is biphasic. An increase in plasma concentrations of testosterone at 45 min after immobilization can be observed, whereas at 180

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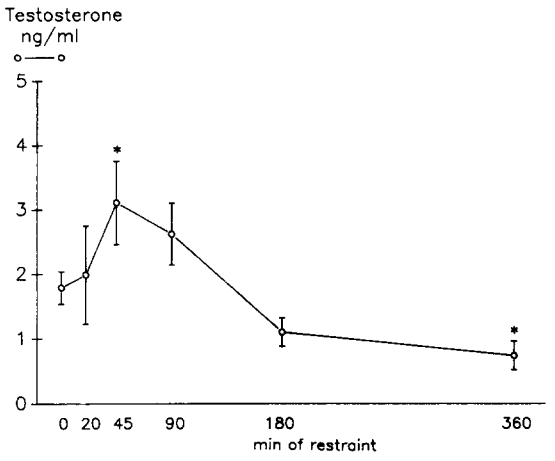

Fig. 1. Changes in concentration of plasma testosterone in adult male Wistar rats during 360 min of immobilization stress. Values are means ± SEM for at least 8 rats per group. *$P < 0.05$ vs unstressed levels (Newman–Keuls test).

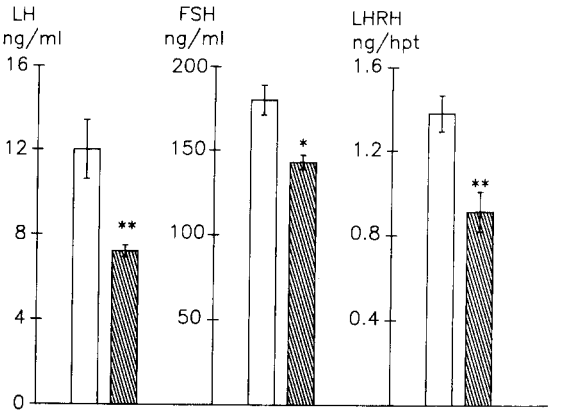

Fig. 3. Effect of chronic restraint (6 h daily over 4 days) on plasma concentration of LH, FSH and hypothalamic LHRH content. Values are means ± SEM for 9 rats per group. *$P < 0.05$, **$P < 0.01$ vs unstressed group (Student's t-test). Stressed animals: ▨, control animals: □.

and 360 min plasma testosterone levels were lower than basal values. Four days of chronic restraint decreased plasma concentrations of testosterone as well as the weights of androgen-dependent organs (Fig. 2).

The inhibitory effect of chronic stress on plasma testosterone secretion is well documented [2–8]. The manner in which stressful stimuli alter testosterone secretion is not yet fully understood. Charpenet *et al.* [19] found that restraint stress induced Leydig cell hyposensitivity to gonadotropin and blockade of testosterone biosynthesis with no alterations in plasma LH levels. However data from our laboratory and others indicate that the effect of restraint stress on testosterone secretion seems to be secondary to modifications in pituitary gonadotropin secretion, since a biphasic secretion of LH during immobilization stress was also observed [20–22] and is also decreased after 4 days of chronic restraint (Fig. 3).

Whether the reduction in gonadotropin secretion is the only factor mediating testosterone reduction after chronic stress remains to be established. Glucocorticoids are triggered by stress and represent an important component of the adaptative response to it. Thus they may exert a modulatory influence upon secretion of testosterone by the testis. Glucocorticoid receptors have been identified in the rat Leydig cell [23]. Moreover glucocorticoids are known to inhibit testosterone synthesis and to reduce the testicular response to HCG, without modifications in basal or LHRH-stimulated gonadotropin levels [13, 14, 24].

To determine the possible role of glucocorticoids in the testosterone response to chronic stress, adult male rats were adrenalectomized

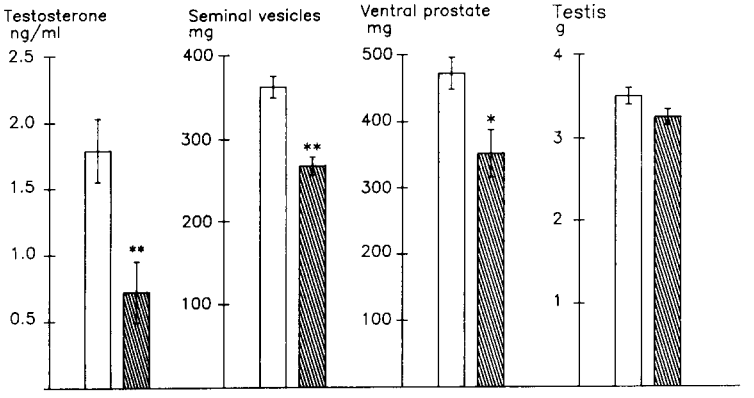

Fig. 2. Plasma concentration of testosterone, seminal vesicles, ventral prostate and testicular weights, in control rats (□) or in rats restrained 6 h daily over 4 days (▨). Values are means ± SEM for at least 7 rats per group. *$P < 0.05$, **$P < 0.01$ vs the unstressed group (Student's t-test).

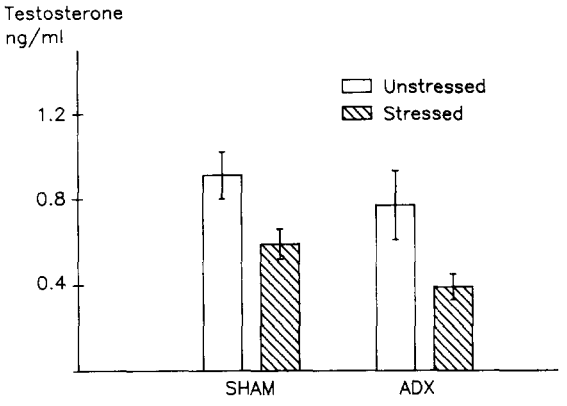

Fig. 4. Effect of adrenalectomy on plasma testosterone response to chronic restraint (6 h daily over 4 days). Values are means ± SEM for 8 rats per group. There was a significant ($P < 0.01$) effect of restraint, but not of adrenalectomy, on plasma concentration of testosterone as revealed by two-way analysis of variance.

under light ether anaesthesia and 4 days later, restrained over 4 consecutive days as described above. In sham operated animals chronic stress evoked a significant decrease in plasma testosterone levels, and adrenalectomy did not prevent the testosterone reduction evoked by restraint (Fig. 4). Our results therefore indicate that the chronic stress-induced decrease in testosterone secretion is not mediated by adrenal secretion. However we found that adrenalectomy totally suppressed the inhibitory effect of chronic stress on prolactin secretion and glucocorticoid administration to adrenalectomized rats reversed this effect [25]. In the adult male rat chronic restraint decreases plasma concentrations of LH and FSH in both intact and adrenalectomized rats [5, 26, 27]. These findings indicate that the adrenal cortex is able to play an inhibitory role on prolactin secretion only through a prolonged release of glucocorticoids. On the contrary the decrease in gonadotropins and testosterone secretion during chronic stress is unlikely to be secondary to the increased adrenal secretion. In support with the hypothesis that the decrease in testosterone secretion is secondary to a decrease in gonadotropin secretion it has been reported that in the hypophysectomized rats, HCG stimulation was able to induce a similar increase in plasma testosterone values in both control and stressed rats [19].

Several studies have shown that episodic LH secretion, including pulse and frequency is greatly reduced during stress [28–30], suggesting that the decrease in LH secretion is centrally mediated. The gonadotropin response to restraint stress might be due to modifications in hypothalamic LHRH secretion, since hypothalamic LHRH content were significantly decreased after 4 days of immobilization (Fig. 3). A decline in hypothalamic LHRH content has also been reported after 3 min of ether exposition [31], but no change in hypothalamic LHRH levels was found after chronic restraint [32]. Some of this discrepancy may be due to the different stress periods used in the experiments.

Although chronic stress decreased gonadotropin secretion, this attenuation in plasma LH and FSH levels was not associated with any decline in pituitary responsiveness to LHRH. Furthermore the decrease in hypothalamic LHRH content is concomitant with an enhanced response of plasma gonadotropin to LHRH stimulation (Fig. 5). An enhanced pituitary response to LHRH has been also observed in man following surgery and in chronically stressed rats [10, 32]. Increased responsiveness to LHRH and decreased LHRH hypothalamic content indicated that the stress-induced decrease in LH, FSH and testosterone secretion was due to the inhibition of LHRH secretion.

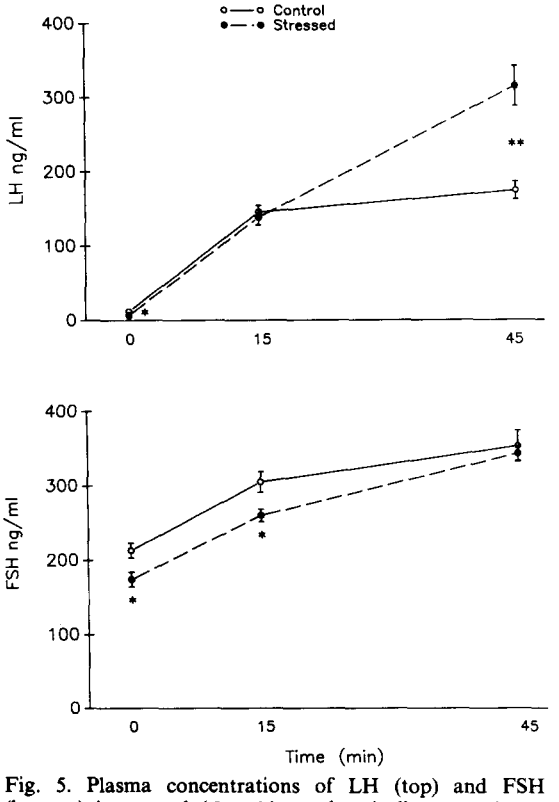

Fig. 5. Plasma concentrations of LH (top) and FSH (bottom) in control (○—○) or chronically stressed rats (●--●), before, 15 and 45 min after an i.p. injection of 500 ng LHRH. Values are means ± SEM for 8 to 10 rats per group. $*P < 0.05$, $**P < 0.01$ vs control group (Student's t-test). (From Ref. [20] with permission).

ROLE OF THE ENDOGENOUS OPIOIDS

The hypothalamic–pituitary–adrenal axis hyperfunction has been postulated as a mechanism for stress-induced reproductive failure [12, 33]. As mentioned above, glucocorticoid secretion during stress appears not to be responsible for the decrease in plasma concentration of LH, since the decline was not reversed by adrenalectomy or metyrapone administration [5, 26, 27, 34], on the contrary dexamethasone administration to adrenalectomized rats prevented the decrease in plasma concentration of gonadotropins after chronic restraint [27]. Another protective effect of glucocorticoids on LH secretion has been recently reported. Glucocorticoid replacement during 7 days prevented the decrease in plasma LH that is observed after CRH administration in the ovariectomized–adrenalectomized rhesus monkey [35]. These data support the hypothesis that reproductive inhibition during chronic stress is probably due to the activation of the hypothalamic–pituitary factors involved in the regulation of the adrenal axis.

Endogenous opioids exert an inhibitory influence on LH secretion by acting on hypothalamic LHRH secretion [36, 37]. Various types of stress have been demonstrated to increase the secretion of the endogenous opioids [38, 39], suggesting that the endogenous opiates may mediate the effect of stress on gonadotropin secretion.

Therefore we analyzed the effect of chronic restraint on β-endorphin content in the hypothalamus, in order to find a possible correlation with the decrease in plasma LH and hypothalamic LHRH levels.

Chronic restraint induced a significant increase in serum β-endorphin whereas no significant modification can be observed either in the hypothalamus or in the pituitary (Table 1). As chronic restraint increases serum β-endorphin levels and adrenal weight, the absence of modifications in pituitary β-endorphin might reflect an increase in both the biosynthesis and the secretion of the peptide. A similar result was obtained by Holtz *et al.* [38], who observed an increase in pituitary β-endorphin content only after 7 days of foot shock stress.

Table 1. Effect of chronic restraint on serum, pituitary and hypothalamic β-endorphin content and on adrenal weight

β-endorphin	Control	Stress
Serum (pg/ml)	215 ± 41	440 ± 68^a
Anterior pituitary (μg)	1.74 ± 0.12	1.53 ± 0.2
Hypothalamus (pg)	13.6 ± 0.87	14 ± 0.73
Adrenal weight (mg)	67 ± 1.7	86 ± 2.3^b

Values are mean $\pm$ SEM $^aP < 0.05$, $^bP < 0.01$ compared to control group.

The effect of stress on hypothalamic POMC derived peptides is controversial, since no changes in β-endorphin was observed after prolonged foot shock or swimming stress [40–42] or in the ACTH concentration after chronic immobilization [43]. It can be argued that the lack of change in the hypothalamic β-endorphin content after chronic stress was due to the fact that both biosynthesis and secretion of this peptide were enhanced. But this does not seem to be the cause, since we have observed that chronic restraint significantly increases POMC mRNA concentration in the anterior pituitary, whereas no changes could be observed in the medial basal hypothalamus (authors, unpublished observations).

Although hypothalamic β-endorphin secretion might not increase during chronic stress, we cannot exclude the fact that pituitary β-endorphin or other hypothalamic opioids play an important role in the stress-induced decrease in gonadotropin secretion.

To study the possible role of endogenous opioids in mediating the testicular axis response to stress, we examined the effect of the opiate receptor antagonist naltrexone, on plasma gonadotropin responses to chronic stress in adrenalectomized rats. Forty rats were adrenalectomized, under light ether anaesthesia and received 0.9% (w/v) NaCl in their drinking water. Four days later 20 rats were injected s.c. with 2 mg naltrexone/kg body wt (naltrexone hydrochloride) or saline 250 μl, at 10.00 and 14.00 h. Half of the animals in each group were stressed as described above. Rats were submitted to chronic stress and naltrexone treatment over 4 days.

Naltrexone treatment during 4 days was not able to modify the decrease in plasma gonadotropin levels after restraint (Fig. 6). The lack of effect of naltrexone in preventing LH and FSH reduction after chronic stress cannot be due to an adaptation of the opioid receptors, since naltrexone treatment at the same dose was able to antagonize the effect of morphine on plasma LH and prolactin levels [44]. These results do not support an essential role for endogenous opioids as mediators in the effects of chronic restraint on gonadotropin secretion.

Some authors have reported that the opioid antagonist naloxone or β-endorphin antiserum or other antagonists, reverse the decrease in

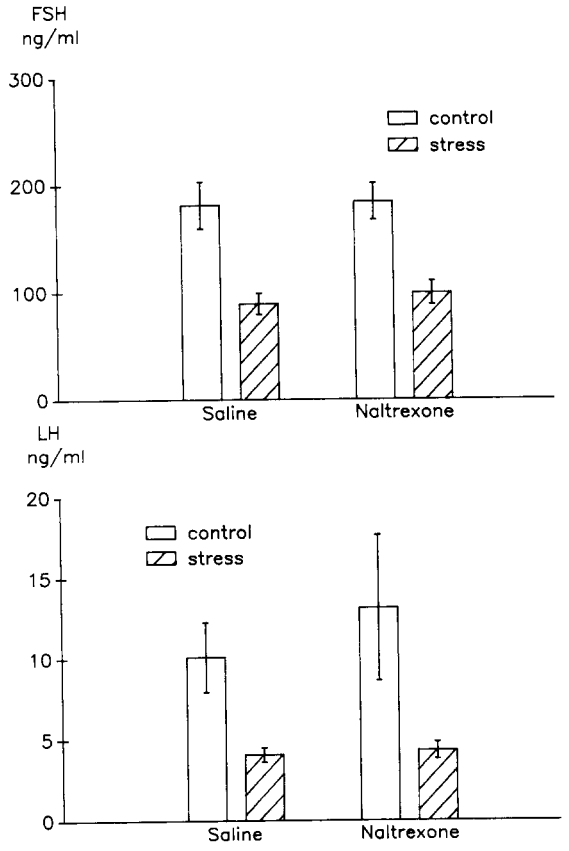

Fig. 6. Effect of naltrexone administration to adrenalectomized rats on plasma FSH (top) or LH levels (bottom) responses to chronic immobilization. Values are means ± SEM for 8 rats per group. Chronic stress decreased plasma concentration of FSH ($P < 0.01$) and LH ($P < 0.01$) as revealed by two-way analysis of variance.

plasma concentration of LH induced by acute stress in the castrated animals [45–48]. Differences between our results and those might reside in the fact that acute stress may act in a different way than chronic stress on the gonadal axis. They performed the experiments in castrated animals instead of intact ones, and castration can modify the gonadotropin response to stress. Indeed acute stress induces an increase in plasma LH levels in the intact rat [20–22, 48, 49], but in the castrated animal an inhibitory effect of acute stress on plasma concentrations of LH has been described [45–47].

One possible explanation is that endogenous CRF is involved in the decrease in LH secretion during stress. The inhibitory effect of CRF on the testicular axis is exerted, like stress, at hypothalamic level, since CRF inhibits the release of LHRH [18, 50], whereas it does not interfere with the LHRH-induced LH release [51]. Furthermore the administration of a synthetic CRF antagonist blocks the inhibitory effect of foot shock stress on the plasma concentration of LH [52].

In conclusion, these data indicate that stressful stimuli may inhibit the testicular axis by acting directly on the hypothalamic LHRH secretion, through some hypothalamic–pituitary components of the adrenal axis other than opioids.

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