Endocrine (1995) 3, 13-20

Effects of corticosterone and testosterone on pituitary gonadotropin content, secretion, bioactivity and messenger RNA levels in the presence or absence of GnRH in male rats

J.M. McAndrews[1], S.J. Ringstrom[1], K.D. Dahl[2], & N.B. Schwartz[1]

[1]*Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois 60208 U.S.A. and* [2]*Department of Medicine, VA Medical Center and University of Washington, Seattle, Washington 98108, U.S.A.*

The effects of corticosterone (B) and testosterone (T) on pituitary and serum bioactive and immunoreactive gonadotropins and on gonadotropin hormone subunit messenger RNA levels were compared in the absence of GnRH. Male rats were implanted with pellets of either cholesterol, B or T. At implantation, 2 and 4 days later half of each group received GnRH antagonist and animals were killed 5 days after implantation. As expected, GnRH antagonist lowered bioactive and immunoreactive serum FSH and LH, pituitary FSH, LHβ and FSHβ mRNA. B treatment alone lowered bioactive and immunoreactive serum FSH and immunoreactive serum LH. B reversed the antagonist effect on bioactive and immunoreactive pituitary FSH and FSHβ mRNA. T alone lowered bioactive and immunoreactive serum FSH and LH levels. T reversed the antagonist effect on bioactive and immunoreactive pituitary FSH. T lowered bioactive and immunoreactive pituitary LH and LHβ mRNA and partially reversed the antagonist effect on FSHβ mRNA. The data suggest that either B or T enhance FSH synthesis by acting directly at the gonadotrope, but that B does not affect LH variables to the same extent as T. The results suggest that in stressed animals, when T levels are reduced, B can substitute for T in sustaining FSH synthesis.

Keywords: gonadotropin; corticosterone; testosterone; GnRH

Introduction

What we asked in the present study was whether glucocorticoids were acting similarly to T by examining the effects of both B and T on various gonadotropin hormone parameters in the same experiment. This is the first study in which high physiological levels of B and T were administered *in vivo* to animals in the same experiment to examine this issue. If both steroids do act similarly, this would indicate an interesting adaptive strategy and suggest that during a time of stress glucocorticoids may substitute for T in the same mechanism. The other possibility is that the two steroids might act by a different mechanism on the same gonadotropes, which would also be of interest.

We have shown under several circumstances that *in vivo* treatment of rats with elevated levels of glucocorticoids: (1) suppresses serum LH, without affecting pituitary levels of LH; (2) elevates pituitary content of FSH without affecting serum FSH and (3) selectively increases pituitary FSHβ mRNA without affecting α or LHβ mRNA (Ringstrom & Schwartz 1985; Suter *et al.*, 1988; Ringstrom *et al.*, 1991; Ringstrom *et al.*, 1992; McAndrews *et al.*, 1994).

Others have shown that testosterone (T) is the major feedback regulator of the gonadotropins in the male rat (Fink, 1988). It has been demonstrated that T is not always a negative feedback regulator of FSH. When GnRH secretion and/or action is blocked, T has a stimulatory effect on FSH, similar to the effects that have been seen with glucocorticoid exposure. Treatment of castrate male rats with T results in: (1) decreases in serum LH and FSH (Spitzbarth *et al.*, 1988); (2) increased pituitary FSHβ mRNA (Wierman *et al.*, 1988; Iliff-Sizemore *et al.*, 1990) and (3) increases in serum FSH when animals are also given a GnRH antagonist (Bhasin *et al.*, 1987). There is also evidence that T may increase FSHβ mRNA stability in male rats (Paul *et al.*, 1990).

Glucocorticoids, as well as other steroid hormones, have distinct biological actions and act via nuclear receptors, which serve as ligand-activated transcription factors (Evans, 1988). The consensus hormone response element (HRE) GGTACAnnnTGTTCT can function as a response element for glucocorticoids, progesterone, androgens and mineralocorticoids (Beato, 1989). Under conditions of elevated glucocorticoids, serum LH declines, and, as a result, serum T is lowered.

The specific objective of the present study was to compare the effects of corticosterone (B), the native glucocorticoid hormone in the rat, and testosterone in the presence or absence of a GnRH signal in male rats. Adult intact male rats were implanted with pellets of either cholesterol, corticosterone, or testosterone, and given injections of oil or GnRH antagonist at 48 h intervals. Treatment with the corticosterone pellets resulted in a serum level of B similar to that seen during times of stress, and the testosterone pellets also delivered a higher than normal but physiological level of serum T. Since GnRH is the only known positive hypothalamic factor controlling FSH, the GnRH antagonist was used to block that effect, although other factors could be involved. Animals were sacrificed after 5 days of treatment and pituitary and serum bioactive and immunoreactive FSH and LH levels and gonadotropin hormone subunit messenger RNA levels were determined.

Correspondence: J.M. McAndrews
Received 6 June 1994; accepted 9 August 1994

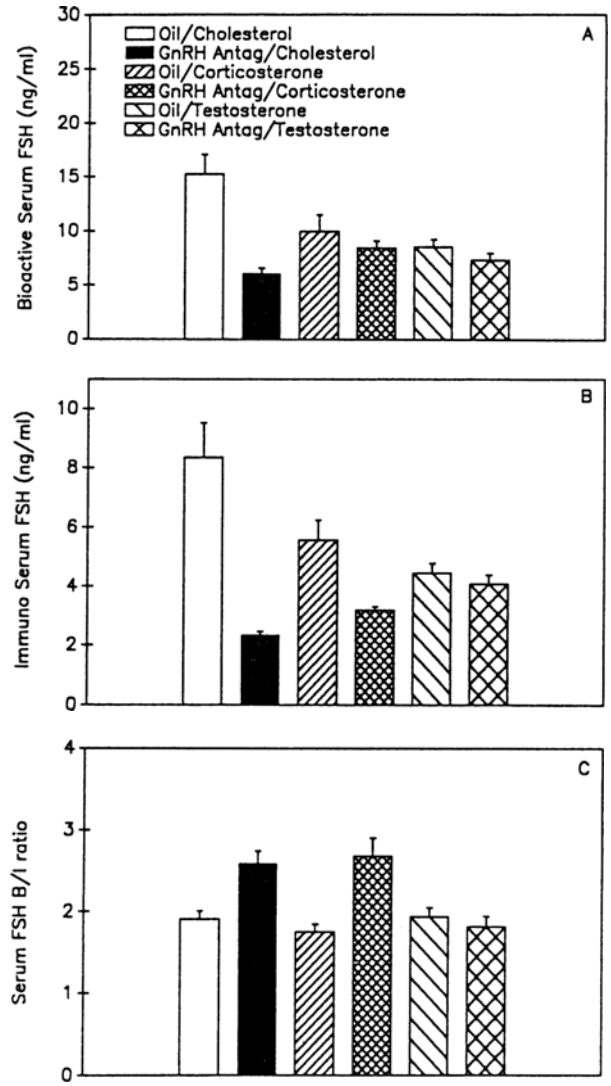

Figure 1 Bioactive serum FSH (**A**), immunoreactive serum FSH (**B**), and serum FSH B/I ratios (**C**) from male rats implanted with either cholesterol, corticosterone, or testosterone pellets and given sc injections of 500 μg GnRH antagonist in oil or oil alone at 48 h intervals beginning at the time of steroid implantation. Animals were killed 5 days after implantation. Each bar represents the mean ±SEM of values from 10 animals. Bioactive serum FSH was significantly lowered by GnRH antagonist treatment ($P<0.001$), B or T treatment ($P<0.05$). There was a significant interaction between GnRH antagonist and B or T treatment ($P<0.001$).Immunoreactive serum FSH was significantly lowered by GnRH antagonist treatment ($P<0.001$), and there was a significant interaction between GnRH antagonist and T treatment ($P<0.001$). The serum FSH B/I ratio was significantly increased by GnRH antagonist treatment ($P<0.001$). There was a significant effect of T treatment ($P<0.05$), and a significant interaction between GnRH antagonist and T treatment ($P<0.001$)

Results

Serum FSH (Figure 1)

Bioactive serum FSH was significantly lowered by GnRH antagonist (Figure 1A). In addition, treatment with either B or T lowered bioactive serum FSH as indicated by a significant effect of steroid treatment. Treatment with either B or T partially reversed the GnRH antagonist effect, indicated by a significant interaction between GnRH antagonist and steroid treatment.

Immunoreactive serum FSH was lowered by treatment with GnRH antagonist (Figure 1B). In addition, treatment with either B or T lowered immunoreactive serum FSH when compared to the oil/C-treated animals. GnRH antagonist treatment further lowered immunoreactive serum FSH in animals treated with B, but not in animals treated with T. This was indicated by a significant interaction between GnRH antagonist and T treatment.

The serum FSH B/I ratio (Figure 1C) was significantly increased by GnRH antagonist treatment alone. Treatment with B or T alone did not change the B/I ratio. However, there was a significant effect of steroid treatment and a significant interaction between GnRH antagonist treatment and steroid treatment, reflecting the fact that T treatment lowered the effect of GnRH antagonist treatment on the B/I ratio.

Serum LH (Figure 2)

Bioactive serum LH (Figure 2A) was significantly lowered by both GnRH antagonist and T treatment, but not by treatment with B. GnRH antagonist treatment also lowered bioactive serum LH in animals treated with B, but did not lower bioactive serum LH further in animals treated with T, indicated by a significant interaction between GnRH antagonist and T treatment.

Immunoreactive serum LH (Figure 2B) was significantly lowered by GnRH antagonist treatment and treatment with either B or T. GnRH antagonist treatment also lowered immunoreactive serum LH in animals treated with B, but not in animals treated with T, indicated by a significant interaction between GnRH antagonist and T treatment.

The serum LH B/I ratio was significantly increased by GnRH antagonist treatment alone (Figure 2C), and by treatment with either B or T.

Pituitary content of FSH (Figure 3)

Bioactive pituitary FSH (Figure 3A) was significantly lowered by treatment with GnRH antagonist alone. Treatment with either B or T completely reversed the suppression by GnRH antagonist, indicated by significant effects of steroid treatment and a significant interaction between GnRH antagonist treatment and steroid treatment.

Immunoreactive pituitary content of FSH (Figure 3B) was significantly lowered by GnRH antagonist treatment, and increased by treatment with B. Treatment with either B or T completely reversed the suppressive effect of GnRH antagonist, indicated by a significant interaction between GnRH antagonist and steroid treatment.

The pituitary FSH B/I ratio (Figure 3C) was lowered by treatment with B.

Pituitary content of LH (Figure 4)

There were no significant effects of GnRH antagonist or B treatment on bioactive pituitary LH (Figure 4A) or immunoreactive pituitary content of LH (Figure 4B). Both bioactive and immunoreactive pituitary LH were significantly suppressed by T treatment.

There were no significant effects of any treatment on the pituitary LH B/I ratio.

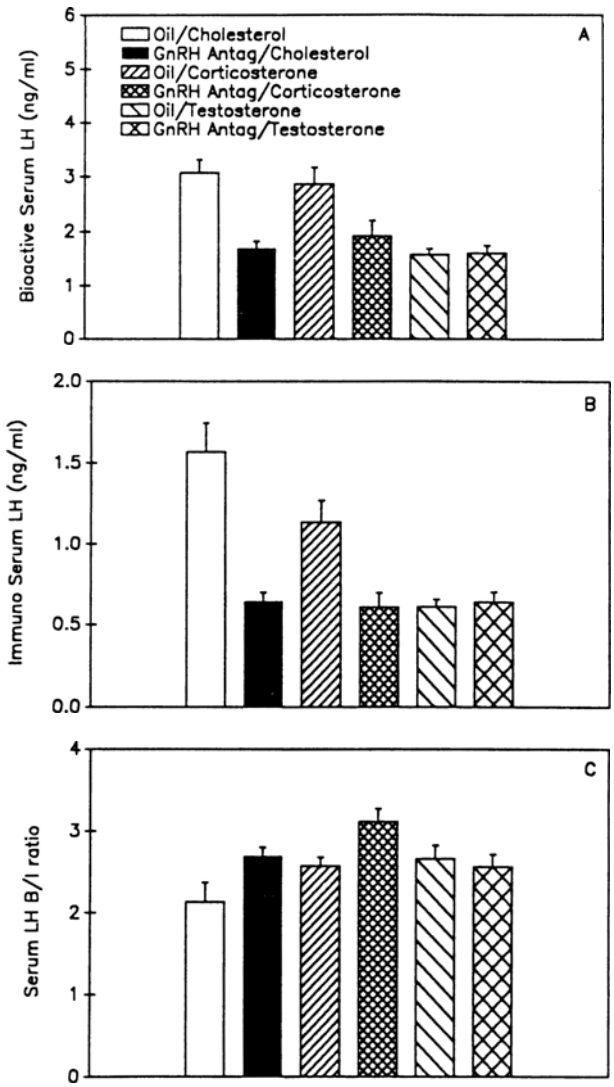

Figure 2 Bioactive serum LH (**A**), immunoreactive serum LH (**B**), and serum LH B/I ratios (**C**) from male rats treated as described in the legend to Figure 1. Each bar represents the mean ±SEM of values from 10 animals. Bioactive serum LH was significantly lowered by GnRH antagonist treatment ($P<0.001$) and treatment with T ($P<0.001$). There was also a significant interaction between GnRH antagonist and T treatment ($P<0.01$). Immunoreactive serum LH was significantly lowered by GnRH antagonist treatment ($P<0.001$) and steroid treatment ($P<0.001$). There was a significant interaction ($P<0.001$) between GnRH antagonist and T treatment. The serum LH B/I ratio was significantly increased by GnRH antagonist treatment alone ($P<0.05$) and by treatment with either B or T ($P<0.05$)

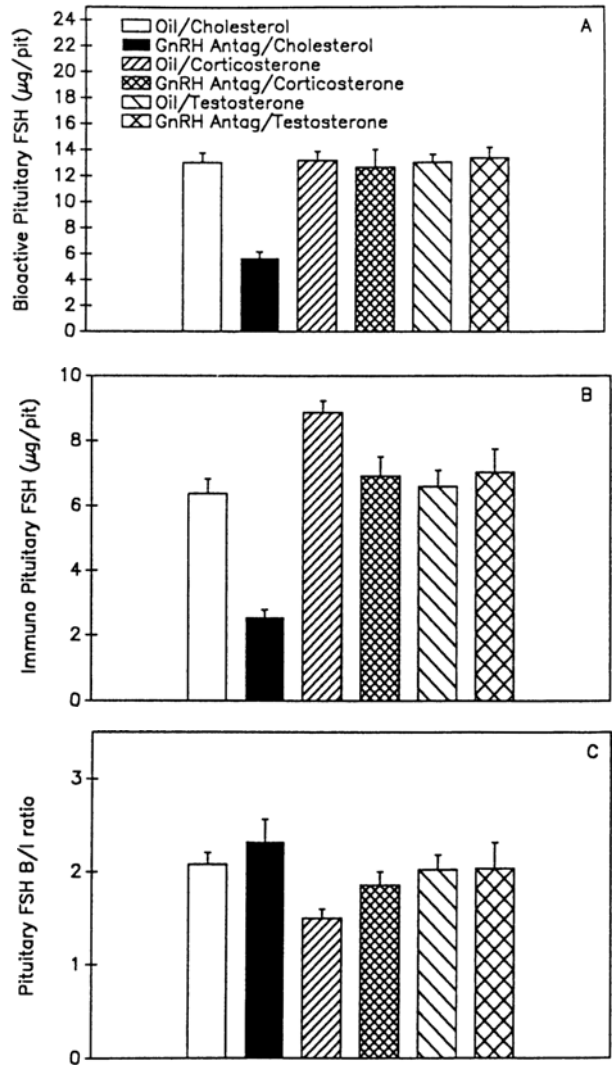

Figure 3 Bioactive pituitary FSH (**A**), immunoreactive pituitary FSH (**B**), and pituitary FSH B/I ratios (**C**) from male rats treated as described in the legend to Figure 1. Each bar represents the mean ±SEM of values from 3–4 animals. Bioactive pituitary FSH was lowered by GnRH antagonist treatment ($P<0.01$). Treatment with either B or T reversed this suppression, indicated by significant effects of steroid treatment ($P<0.001$) and a significant interaction between GnRH antagonist and steroid treatment ($P<0.001$). Immunoreactive pituitary content of FSH was significantly lowered by GnRH antagonist treatment ($P<0.05$). Treatment with B significantly increased immunoreactive pituitary content of FSH ($P<0.01$), and there was a significant interaction ($P<0.05$) between GnRH antagonist and steroid treatment. The pituitary FSH B/I ratio was lowered by treatment with B ($P<0.05$)

Gonadotropin hormone subunit messenger RNA levels (Figures 5 and 6)

A representative Northern blot is shown in Figure 5, and the combined data obtained by quantitative analysis is presented in Figure 6.

Treatment with GnRH antagonist significantly suppressed FSHβ mRNA (Figure 6A) as did treatment with T. B treatment alone had no effect. GnRH antagonist treatment did not lower FSHβ mRNA in the presence of B, and the suppressive effect of the antagonist on T-treated animals was not as profound as that seen in C-treated controls, indicated by a significant interaction between steroid treatment and GnRH antagonist treatment.

There was no significant effect of GnRH antagonist or B treatment on LHβ mRNA (Figure 6B). T treatment significantly lowered LHβ mRNA in the presence or absence of a GnRH signal.

There were no significant effects of any treatment on α mRNA levels (Figure 6C).

Discussion

An exciting finding from the present study was the demonstration that the two steroids (corticosterone and testosterone) could affect LH and FSH differently,

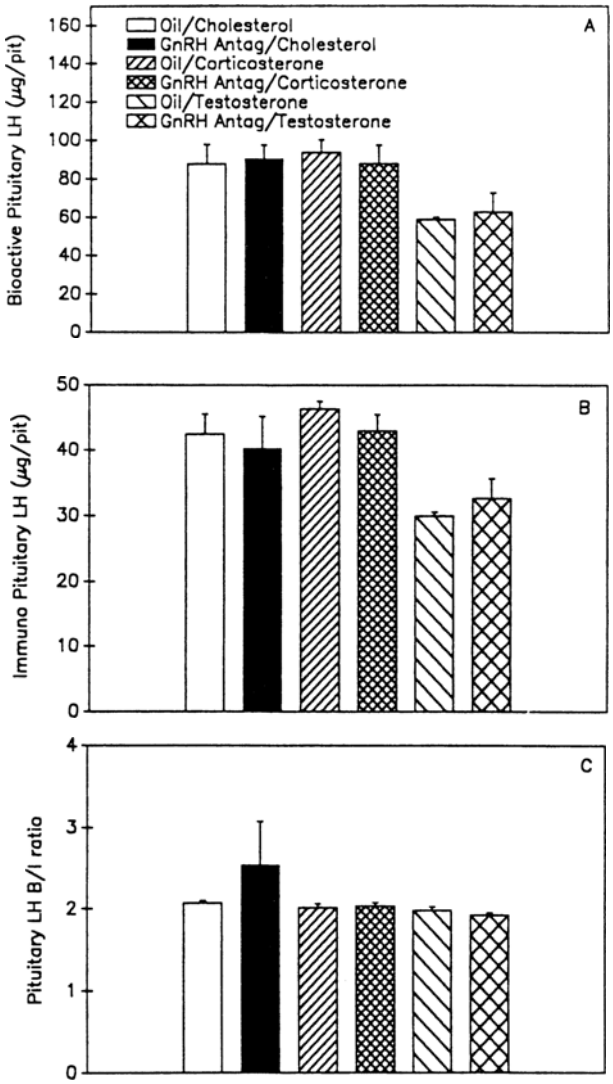

Figure 4 Bioactive pituitary LH (**A**), immunoreactive pituitary LH (**B**), and pituitary LH B/I ratios (**C**) from male rats treated as described in the legend to Figure 1. Each bar represents the mean ±SEM of values from 3–4 animals. Both bioactive and immunoreactive pituitary content of LH was significantly lowered by T treatment ($P<0.01$ and $P<0.05$, respectively). The pituitary LH B/I ratio was not affected by any treatment

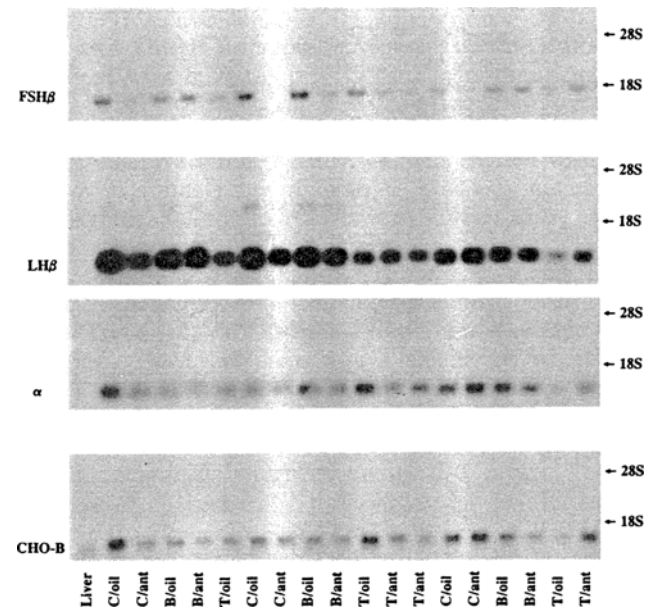

Figure 5 Northern blots of FSHβ (top row), LHβ (2nd row), α (3rd row) and CHO-B (bottom row) transcripts from pituitaries of animals as described in the legend to Figure 1. RNA was isolated from individual pituitaries (lanes 2–19) and from rat liver (lane 1; used as a negative control). 10 μg of each RNA sample were separated on denaturing, agarose-formaldehyde gels and transferred to nylon membrane. The membrane was first probed with radiolabeled genomic rFSHβ DNA insert and exposed to film for 55 h. The same blot was then sequentially probed with radiolabeled rLHβ, rα and CHO-B and exposed to film for 120 h, 45 h and 120 h respectively. The CHO-B probe was used to assess RNA concentration in each lane. The size markers represent 28S and 18S ribosomal subunit RNAs. (C=cholesterol, B=corticosterone, T=testosterone and ant=GnRH antagonist)

presumably in the same gonadotrope. Both corticosterone and testosterone showed similar effects on FSH parameters. The results suggest that both B and T may be acting on FSH synthesis, since B and T were both able to reverse the effects of the GnRH antagonist on bioactive and immunoreactive pituitary FSH. In contrast, testosterone affected LH parameters differently from corticosterone in some instances. Since it is known that FSH and LH are synthesized in the same cells, our findings represent an interesting example of differential regulation of FSH and LH.

The overall results of this study are summarized in Table 1. Treatment with either B or T in conjunction with the GnRH antagonist resulted in similar effects with respect to FSH parameters. The effects of B and T on pituitary FSH and FSHβ mRNA in the presence of the GnRH antagonist show direct effects at the level of the pituitary, independent of GnRH input. The GnRH antagonist was used as a tool to determine these direct effects. In the presence of the GnRH antagonist, B treatment completely reversed the suppressive effects of the antagonist on bioactive and immunoreactive pituitary FSH, and FSHβ mRNA. The consensus, (Suter & Schwartz 1985a; Suter & Schwartz 1985b; Kamel & Kubajek 1987; Baldwin *et al.*, 1991; Ringstrom *et al.*, 1991; Ringstrom *et al.*, 1992; McAndrews *et al.*, 1994) based on a large body of evidence, is that the major effect of glucocorticoids is at the level of the gonadotrope by increasing synthesis or blocking intracellular degradation of FSH. Similarly, in the presence of GnRH antagonist, treatment with T completely reversed the suppressive effects of the antagonist on bioactive and immunoreactive pituitary FSH. It seems probable that T is also acting directly at the level of the gonadotrope in reversing the suppressive effects of the GnRH antagonist on bioactive and immunoreactive pituitary FSH in our experiment. There is also evidence that T may increase FSHβ mRNA stability in male rats (Paul *et al.*, 1990). In addition, treatment with either B or T in the presence of the GnRH antagonist resulted in a slight reversal of the antagonist effect on bioactive and immunoreactive serum FSH.

Several androgen-specific effects on LH parameters were noted in this study (Table 1), confirming those reported by other. Treatment with T lowered bioactive serum LH, while treatment with B alone had no effect.

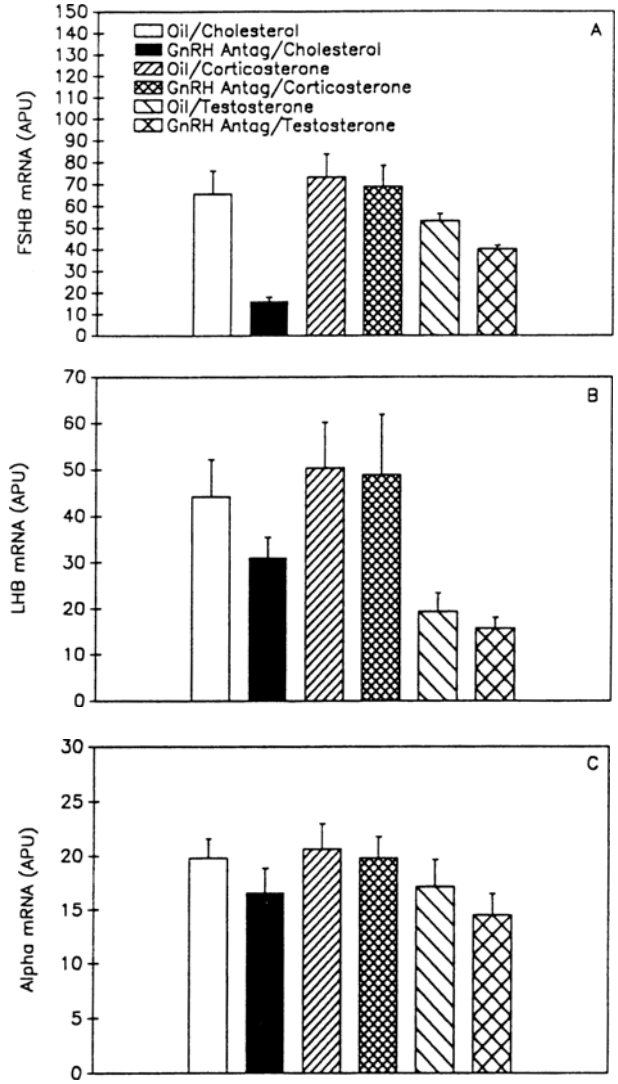

Figure 6 FSHβ (**A**), LHβ (**B**) and α (**C**) messenger RNA levels in pituitaries from male rats treated as described in the legend to Figure 1. Gonadotropin subunit messenger RNAs were measured in individual pituitaries by Northern blot hybridization analysis. Each bar represents the mean ±SEM of values from 6 autoradiographic bands, as measured by phosphorimaging, and standardized by dividing the values for the subunits by the values for CHO-B. Treatment with GnRH antagonist significantly lowered ($P<0.001$) FSHβ mRNA as did T treatment ($P<0.001$). There was also a significant interaction ($P<0.01$) between GnRH antagonist and steroid treatment. Treatment with T significantly ($P<0.001$) lowered LHβ mRNA. There were no significant effects of any treatment on α mRNA

Table 1 Summary of corticosterone and testosterone effects

	Compared to oil/cholesterol			*Compared to antagonist/cholesterol*	
	Antag/Chol	*Oil/B*	*Oil/T*	*Antag/B*	*Antag/T*
Bioactive serum FSH	↓	↓	↓	Slight ↑	Slight ↑
Immuno serum FSH	↓	↓	↓	Slight ↑	Slight ↑
Bioactive serum LH	↓	–	↓	–	–
Immuno serum LH	↓	↓	↓	–	–
Bioactive pituitary FSH	↓	–	–	↑	↑
Immuno pituitary FSH	↓	↑	–	↑	↑
Bioactive pituitary LH	–	–	↓	–	↓
Immuno pituitary LH	–	–	↓	–	↓
FSHβ mRNA	↓	–	↓	↑	↑
LHβ mRNA	–	–	↓	–	↓
α mRNA	–	–	–	–	–

Antag = GnRH antagonist; Chol = cholesterol; B = corticosterone; T = testosterone.

This finding is similar to that seen in normal men following chronic administration of high doses of T (Dahl & Stone, 1992). In a study using dispersed male rat pituitary cell cultures, it was found that T suppressed the glycosylation of LH α-subunit (Krummen & Baldwin, 1988). A decrease in bioactive serum LH might be the result of a reduction in the amount of LH, or, alternatively, it is possible that T may be affecting the glycosylation of the secreted LH hormone, as has been shown previously. In this study, glucocorticoid treatment did not alter immunoreactive pituitary content of LH, as we have seen previously in other studies (Ringstrom & Schwartz 1985; Suter *et al.*, 1988; Ringstrom *et al.*, 1991; McAndrews *et al.*, 1994). In contrast, treatment with T did significantly suppress immunoreactive pituitary content of LH. Whether T is acting at the level of the hypothalamus or the anterior pituitary gland, or acting at both sites to decrease immunoreactive pituitary LH content is not clear from this study. That T acts primarily at the level of the hypothalamus to inhibit GnRH secretion and, in turn, inhibit gonadotropin synthesis and secretion can be inferred from data obtained from male rats (Steiner *et al.*, 1982). Treatment with T also lowered LHβ mRNA in this study, while treatment with glucocorticoids had no effect, confirming the results from other studies using male and female rats (Ringstrom *et al.*, 1991; McAndrews *et al.*, 1994). Other studies performed on castrate male rats have shown that T replacement also decreases α subunit mRNA, reviewed in (Gharib *et al.*, 1990). We did not observe any suppression of α subunit mRNA by T treatment. A recent study using intact male rats found that T treatment did lower α subunit mRNA (Perheentupa *et al.*, 1993). However, not only was a higher dose of T used compared to our study, but the animals were treated for a longer period of time (2 weeks), which may explain the differences between the two studies. Treatment with T lowered bioactive pituitary LH, while treatment with B had no effect. The decrease in bioactive pituitary LH might be the result of a suppression in the amount of LH or perhaps T may be affecting the glycosylation of LH stored in the pituitary gland, and thereby affecting its measured biological activity.

It is well established that exposure to a variety of stressors, both natural and experimental, can disrupt reproductive functions in many species (Christian, 1975; Moberg, 1987). Glucocorticoid hormones are produced and secreted by the adrenal cortex in response to stress. There is an abundant literature on the suppressive effects of glucocorticoids on reproductive processes, as reviewed (Moberg, 1987). The inhibitory effects of glucocorticoids of LH secretion *in vitro* (Kamel & Kubajek, 1987; D'Agostino *et al.*, 1990;

Baldwin *et al.*, 1991) suggest that the secretion of LH can be directly affected by glucocorticoids at the pituitary level. The major action of glucocorticoid treatment of LH, regardless of the site of action, seems to be in blocking release of LH. In the human male with elevated glucocorticoids, this suppression of serum LH leads to a suppression of serum testosterone and an impairment of reproductive function (McKenna *et al.*, 1979). Glucocorticoid treatment has also been shown to directly inhibit testicular LH receptors and steroid production in the rat (Bambino & Hsueh, 1981). It has also been established that corticotropin releasing factor (CRF) is released during stress (Plotsky & Vale, 1984) and the anatomical proximity of CRF- and GnRH-secreting neurons in the hypothalamus (MacLusky *et al.*, 1988) suggested the possible involvement of endogenous CRF as a mediator of stress-induced inhibition of reproductive processes. It was subsequently shown that injection of CRF into the third ventricle of female rats inhibited GnRH release into the hypophysial-portal blood (Petraglia *et al.*, 1987). Furthermore, injection of a CRF antagonist into the brain ventricles of castrated male rats reversed the inhibitory action of stress on the secretion of LH (Rivier *et al.*, 1986).

In the present study, in the presence of GnRH antagonist, treatment with B partially reversed the suppressive effects of GnRH antagonist treatment on bioactive serum FSH. This finding is in contrast with a corresponding female study (McAndrews *et al.*, 1994), in which treatment with B specifically increased bioactive serum FSH and completely reversed the suppressive effects of GnRH antagonist treatment on bioactive serum FSH. Increases in bioactive serum FSH with B alone were not seen in the present study, in fact, decreases were observed.

An interesting observation from the present study is the fact that both B and T completely reversed the suppressive effects of the GnRH antagonist on bioactive and immunoreactive pituitary FSH. These effects were also observed with B treatment on bioactive serum FSH and FSHβ mRNA in the previous study using female rats (McAndrews *et al.*, 1994). Whether this is due to a direct effect of the steroids on the gene for the rFSHβ subunit itself remains to be determined. It is known that the consensus hormone response element GGTACAnnnTGTTCT can function as a response element for glucocorticoids, progesterone, androgens, and mineralocorticoids (Beato, 1989). Glucocorticoids and androgens are believed to act by binding distinct receptors, which serve as ligand-activated transcription factors (Evans, 1988). How, then, can androgens and glucocorticoids have distinct effects on target cells since they recognize a common HRE? This could probably be accomplished by the differential expression of the androgen receptor and glucocorticoid receptor in different cell types and the interaction of these receptors with other specific transcription factors. Indeed, this has recently been shown to be true for androgen-specific gene activation (Adler *et al.*, 1992). Transcription response to androgens, and lack of response to glucocorticoids was possible due to nonreceptor factor interactions, and not the distinct receptor DNA binding site.

In summary (Table 1), in the present study T affected some measured variables differently than B, notably lowering bioactive and immunoreactive pituitary LH and LHβ mRNA. In contrast, in several instances B and T appeared to have similar effects, particularly in the presence of the GnRH antagonist, on bioactive and immunoreactive pituitary FSH and on FSHβ mRNA. That B and T have similar effects on pituitary FSH represents a possible important adaptive significance. Since T is low during a time of stress, probably due to low serum LH levels, B serves to 'assume' the role of T during stress. We have consistently observed in male and female rats treated *in vivo* with glucocorticoids that release of LH is inhibited while pituitary content of FSH and FSHβ mRNA is increased. The increased synthesis and storage of FSH in the pituitary gland during a time of stress may prepare the animal for rapid resumption of reproductive functions, particularly gametogenesis, once the stress subsides.

Materials and methods

Animals

Male Sprague-Dawley rats, 180–200 g in weight, were purchased from Charles River (Portage, MI) and housed in facilities approved by the American Association for Accreditation of Laboratory Animal Care in a light-dark cycle of 14L:10D, with water and food provided *ad libitum*. The experimental protocol was approved by the Northwestern University Institutional Animal Care and Use Committee (IACUC).

Protocol

Male rats were implanted subcutaneously with cholesterol or steroid pellets under light metofane (Methoxyflurane, Pitman-Moore, Mundelein, IL) anesthesia at 0800 h on day 1 of the experiment. Animals received either one 400 mg pellet of cholesterol (C), two 400 mg pellets of corticosterone (B).

Table 2 General effects of steroid treatment and GnRH antagonist treatment

	Serum B (μg/dl)	*Paired adrenal weight* (mg)	*Percent body wt gain/loss*	*Serum T* (ng/ml)	*Seminal vesicle weight* (mg)
Oil/Chol	14.23±5.49	54.79±3.34	+10.93±1.8	2.82±0.46	472.8±34.6
GnRH Antag/Chol	10.46±2.34	53.82±4.80	+10.34±0.29	0.1±0.00	261.7±42.3
Oil/B	57.37±5.01*	31.45±1.75*	−17.88±2.7*	0.535±0.24†	270.6±26.5†
GnRH Antag/B	55.62±5.69*	33.12±1.38*	−17.46±2.6*	0.1±0.00†	216.7±13.6†
Oil/T	5.87±1.47	58.69±2.85	+9.37±2.3	4.92±0.53‡	718.1±28.9‡
GnRH Antag/T	9.27±2.7	55.42±3.75	12.19±1.2	3.99±0.35‡	768.4±45.3‡

*Significantly different from cholesterol- and testosterone-treated animals ($P<0.00001$). †Significantly different from cholesterol-treated animals ($P<0.005$). ‡Significantly different from cholesterol-treated and corticosterone-treated animals ($P<0.0001$)

(This dose resulted in a 'physiological' stress level of serum corticosterone; see Table 2), or one 400 mg 1:3 Testosterone:Cholesterol (T) pellet, which delivered an appropriate high physiological level of testosterone as measured in the serum (See Table 2). At the same time, half of each implant group was given 500 μg of GnRH antagonist [WY 45760 ([Ac-β(2)-D-Nal[1], 4-F-D-Phe[2], D-Trp[3], D-Arg[6]] LHRH), Wyeth-Ayerst Research, Princeton, NJ] suspended in sesame oil as an sc injection. The other half of each group received injections of sesame oil vehicle alone. This 500 μg dose of GnRH antagonist was selected for its ability to successfully inhibit immunoreactive serum LH in a previous study (Kartun & Schwartz, 1987) using male rats. This dose is higher than that used in our previous study with female rats (McAndrews *et al.*, 1994). The GnRH antagonist and control injections were given at 48 h intervals at 0800 h beginning at the time of steroid implantation and continuing until the animals were killed at 0800 h on day 6, 5 days after the day of implantation. There were 10 animals per treatment group. Pituitaries were collected at the time of sacrifice, snap frozen, and stored at −70°C until measurement of LH and FSH content (N=4) or gonadotropin subunit mRNA levels (N=6). Serum was stored at −20°C for subsequent hormone measurement by RIA and bioassay.

At the time of sacrifice, the adrenal glands and seminal vesicles were removed from each animal, dissected and weighed and body weight was monitored for each animal. The measurements are summarized in Table 2. Animals which were treated with B had significantly lowered paired adrenal weights, as expected, than either the C-treated group or the T-treated animals. In addition, animals which received B pellets lost a significant percentage of body weight during the experiment when compared to C- and T-treated animals, both of which showed weight gains. Seminal vesicle weight was significantly elevated in animals which received T treatment, as expected.

Pituitaries were homogenized in 0.01 M phosphate buffer with 0.15 M NaCl and 1% (wt/vol) PBS-egg white, pH 7.0, with 1% (vol/vol) Triton X-100 added as solvent (Spitzbarth *et al.*, 1988; Suter *et al.*, 1988). The homogenates were then assayed for LH and FSH by RIA and bioassay.

Radioimmunoassays

LH: Concentrations of LH in serum and pituitary homogenates were measured using an ovine:rat RIA, employing NIH S-26 as a standard and LH S-10 antirat antibody. The mean intraassay coefficient of variation (CV) for LH was 8%.

FSH Concentrations of FSH were measured in the same samples using the rat FSH kit from the National Hormone and Pituitary Distribution Program. NIH-FSH-RP-2 standard and FSH S-11 antirat antibody were used. The mean intraassay CV for FSH was 5%.

Corticosterone Corticosterone was measured in serum using the Immuchem™ Double Antibody Corticosterone RIA kit for rats and mice (ICN Biomedicals, Costa Mesa, CA). The intraassay CV for B values was 1%.

Testosterone Concentrations of testosterone in serum were measured with the RSL ^{125}I Testosterone kit (ICN). The mean intraassay CV for testosterone was 2%.

Bioassays

Bioactive FSH and LH levels in serum and pituitary samples were measured using the *in vitro* rat granulosa cell (Dahl *et al.*, 1989) bioassay and a mouse Leydig tumor cell (Dahl & Sarkissian, 1993) bioassay. The rat FSH preparation RP-2 and LH preparation NIH S-26 used as the reference standards for the bioassays were obtained from the National Pituitary and Hormone Distribution Program. The intra- and interassay variabilities were 10% and 14%, and 8% and 11% respectively, for the FSH and LH bioassays.

Measurement of gonadotropin subunit messenger RNAs

Total RNA was extracted individually from six pituitaries from each experimental group and from rat liver using the cesium chloride gradient centrifugation method (Glisin *et al.*, 1974; Chirgwin *et al.*, 1979). Rat liver tissue was included as a negative control. Ten micrograms (A_{260}) of total RNA from each sample was separated by electrophoresis on a 1% agarose-formaldehyde gel, diffusion-blotted onto nylon membrane (ICN) and covalently attached by UV cross-linking. Blots were sequentially hybridized to the FSHβ, LHβ and α probes. Rat α (Godine *et al.*, 1982), and LHβ (Chin *et al.*, 1983) cDNAs and rat FSHβ genomic DNA (Gharib *et al.*, 1989) probes were the gift of Dr William Chin (Brigham and Women's Hospital, Boston, MA). All probes were radiolabeled by random priming using a kit (Promega, Madison, WI). Specific activity of the probes was $0.75-1.0 \times 10^9$ c.p.m./μg DNA. Blots were then rehybridized to a cDNA clone CHO-B (Harpold *et al.*, 1979), which detects the LLRep3 gene family (Heller *et al.*, 1988), to assess the amount of RNA present in each lane. Blots were stripped between hybridizations with boiling 0.1% sodium dodecyl sulfate. Blots were subjected to both standard autoradiography and phosphorimaging (Molecular Dynamics, Sunnyvale, CA) following each hybridization. Quantification for each hybridized band for each probe was performed by phosphorimaging. The amounts of each gonadotropin mRNA subunit were internally standardized by dividing the values for the subunit by the value for CHO-B in each lane. All mRNA data points represent the means of normalized values ±SEM in arbitrary phosphorimager units (APU).

Statistical Analysis

All results are expressed as the mean ±SEM. The effects of steroid and GnRH antagonist treatment on the measured variables were compared by two-way analysis of variance using CRISP statistical software (CRUNCH Software, San Francisco, CA). *Post-hoc* comparisons were performed using Scheffe's test to reveal significant differences between levels of the between-subject factors. Specific *P* values for the statistical analyses are presented in the figure legends.

Acknowledgements
The authors thank Dr William Chin for the gift of the gonadotropin subunit probes. The technical assistance of Brigitte Mann, Bernadette Meberg, Rachele Kardon and Karla Loken, and the secretarial assistance of Monika Leopold is greatly appreciated. The authors also thank Wyeth-Ayerst Research (Princeton, NJ) for the generous gift of the GnRH antagonist, and the National Hormone and Pituitary Distribution Program for the gonadotropin RIA standards and antibodies. This work was supported in part by NIH grants R01 HD07504, P30 HD28048, P01 HD21921 and T32 HD07068 (to N.B.S.) and the Office of Research Development, Medical Research Service, Department of Veterans Affairs (to K.D.D.).

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