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A Suppression of Gonadotropin Secretion by Cortisol in Castrated Male Rhesus Monkeys (*Macaca mulatta*) Mediated by the Interruption of Hypothalamic Gonadotropin-releasing Hormone Release[1]

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ABSTRACT

Four orchidectomized rhesus monkeys (3–3.5 yr of age) were treated for 62 days with daily i.m. injections of hydrocortisone acetate (HCA) at a dose of 10–20 mg/(kg BW·day), and blood samples were obtained daily or every other day before, during, and after treatment. Hydrocortisone acetate injections resulted in a progressive rise in mean plasma cortisol from basal concentrations of 17–35 μg/100 ml prior to initiation of steroid treatment to approximately 150 μg/100 ml 5 wk later. When serum cortisol concentrations reached 100 μg/100 ml, 3–4 wk after the initiation of HCA treatment, circulating luteinizing hormone (LH) and follicle-stimulating hormone (FSH) began to decline, reaching nondetectable concentrations 35 days later. Withdrawal of HCA resulted in a return in plasma cortisol concentrations to pretreatment control levels, which was associated with a complete restoration of gonadotropin secretion. In 2 animals, administration of an intermittent i.v. infusion of gonadotropin-releasing hormone (GnRH) (0.1 μg/min for 3 min once every hour), which appears to stimulate the gonadotropes in a physiologic manner, reversed the cortisol-induced inhibition of gonadotropin secretion, restoring circulating LH and FSH concentrations to within 80–100% of control. These results suggest that, in the rhesus monkey, the major site of the inhibitory action of cortisol on gonadotropin release resides at a suprapituitary level and is mediated by interruption of hypothalamic GnRH release.

INTRODUCTION

In the course of a study of the role of the adrenal gland in determining the ontogeny of gonadotropin secretion in the rhesus monkey, it was observed that high levels of circulating cortisol, produced accidentally during corticosteroid replacement in one adrenalectomized-orchidectomized infantile animal, were associated with a striking reduction in plasma luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations (Plant and Zorub, 1984). The magnitude of this decline in circulating gonadotropin levels suggested a complete inhibition of open-loop LH and FSH secretion, similar to that previously reported to occur following the abolition of gonadotropin-releasing hormone (GnRH) release that was occasioned by placement of radiofrequency lesions in the mediobasal hypothalamus of castrated adult rhesus monkeys (Plant et al., 1978). The purpose of the present study was to further examine in additional animals this unexpected effect of cortisol and, in the event that the original observation was confirmed, to determine the site of the inhibitory action of this adrenal steroid on LH and FSH release.

MATERIALS AND METHODS

Animals

Four male rhesus monkeys, which were orchidectomized prepubertally, were studied when they were 3.0–3.5 yr of age. Animals were housed individually under a controlled photoperiod (lights on 0600–1800 h) as described previously (Krey et al., 1975).

Catheterization Procedure

In order to infuse GnRH without restraint or tranquilization, monkeys were implanted via an internal jugular vein with an indwelling cardiac catheter made from 19-g polyvinyl chloride tubing and

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[2] Reprint requests.

housed in remote sampling cages as described earlier (Plant, 1981, 1982). Three weeks were allowed for the animals to adapt to the remote sampling cages before experiments were initiated.

Hormone Assays

Luteinizing hormone was estimated by a cynomolgus LH-anti-hCG (rabbit 13, Pool D) radioimmunoassay (RIA) system that employs rhesus pituitary preparation WP-XV-20 (NICHD-rhLH) as a standard (Peckham and Tontala, 1981). This assay, which does not cross-react with LH-like material (Peckham et al., 1977) in the circulation of rhesus monkeys (Peckham and Tontala, 1981), is now distributed as a RIA kit by the National Hormone and Pituitary Program. The sensitivity of the LH assay ranged from 25 to 30 ng WP-XV-20/ml serum. Follicle-stimulating hormone was determined by a hFSH (NIH-FSH-HS-1)-anti-hFSH (Batch 5, NIAMDD-NPA) RIA system, which employs a rhesus pituitary preparation (WP-XIII-21-42) as a standard (Plant and Dubey, 1984). The sensitivity of the assay was 5 ng WP-XIII-21-42/ml plasma.

Circulating cortisol levels were measured by a previously described RIA (Plant and Zorub, 1984). Thyroxine (T_4) concentrations were measured in 10-μl aliquots of serum using a commercially available RIA kit (Gammacoat) from Travenol-Genentech Diagnostics (Cambridge, MA). Ten-microliter aliquots of T_4-free serum from thyroidectomized rhesus monkeys did not displace ^{125}I-T_4 tracer from rabbit anti-T_4 serum. The recovery of known amounts of authentic L-thyroxine (Sigma Chemical Co., St. Louis, MO) added to T_4-free rhesus serum was quantitative (Fig. 1) and displacement curves obtained with serum pools from euthyroid rhesus monkeys were parallel to the standard curve (Fig. 1).

Hormone Treatments

Sustained elevations in plasma cortisol were produced by daily i.m. injections of a suspension of hydrocortisone acetate (HCA) in 0.9% saline (50 mg HCA/ml) either obtained as the commercially available preparation (hydrocortone acetate; Merck Sharpe & Dohme, Westpoint, PA) or prepared in this laboratory from HCA powder generously provided by Merck Sharpe and Dohme. The dose of HCA injected was 10–20 mg/(kg BW•day).

Stock solutions of synthetic GnRH (1 mg/ml) were prepared as described earlier (Plant, 1982). These were diluted to 0.308 μg/ml with 0.9% saline and stored at −20°C until use. The working dilution of GnRH was intermittently infused via the cardiac catheter at 0.33 ml/min (i.e., 0.1 μg GnRH/min) for 3 min every hour using peristaltic pumps (Minipuls 2; Gilson International, Middleton, WI) programmed by an electronic timer (Chrontrol Model DL; Lindberg Enterprises, Inc., San Diego, CA). In orchidectomized males bearing hypothalamic lesions that abolish endogenous GnRH secretion, such a regimen of intermittent GnRH administration appears to provide the gonadotropes with a hypophysiotropic stimulus comparable to that generated by an intact central nervous system (Plant and Dubey, 1984).

Experimental Protocol

In the first experiment, the four animals were each treated for 62 days with HCA. The initial dose was 10 mg HCA/(kg BW•day) but this was subsequently increased after 7 days to 20 mg/(kg BW•day). Time courses of circulating LH and FSH concentrations were monitored throughout the experiment in sera obtained from blood samples (2–3 ml) collected daily or every other day by femoral venipuncture, usually between 0930 and 1130 h. Body weight was determined at the time of venipuncture.

The results of the foregoing experiment confirmed our original observation that elevated levels of plasma cortisol suppressed gonadotropin secretion. A second experiment was therefore conducted in order to determine the site (pituitary versus hypothalamus) of this inhibitory action of cortisol on LH and FSH release. Two of the four monkeys were implanted with a cardiac catheter 7 mo after the completion of the first experiment, and subsequently housed in remote sampling cages. Treatment with HCA [20 mg/(kg BW•day)] was initiated approximately 4 wk later and maintained for 92 days. In order to administer i.m. injections of HCA in this experiment, the animals were lightly sedated with an i.v. bolus of thiamylal sodium (26–29 mg/monkey), an ultrashort-acting barbiturate (Bio-tal; Bio-Ceutic Laboratory Inc., St. Joseph, MO). Injections of vehicle were administered in a similar fashion for 8–10 days before the initiation of HCA treatment and for 56 days after its withdrawal. Blood samples were collected daily, usually between 0930 and 1130 h, via the cardiac catheter. On occasion it was not possible to withdraw blood from the cardiac catheter and samples were then collected by femoral venipuncture at the time of the HCA injection. Serum LH and FSH concentrations in selected samples were monitored once every 1–2 wk in order to track the suppression of gonadotropin secretion. When serum LH was no longer detectable by RIA, an intermittent i.v. infusion of GnRH was initiated (on Day 77 of corticosteroid treatment) and continued for 10 days while maintaining the daily HCA injections. An intermittent i.v. infusion of vehicle (0.9% saline) was continued for 4 days after the termination of GnRH treatment. Body weight was determined at weekly intervals.

Statistical Analysis

In order to evaluate the significance of hormonal changes in Experiment 1, weekly mean concentrations of serum cortisol, LH, and FSH were first calculated. The significance of differences between these means were then determined using an analysis of variance for repeated measures in conjunction with the Student-Newman-Keuls test (Winer, 1962). Correlations between changes in circulating cortisol and gonadotropin concentrations were evaluated with regression analysis (Winer, 1962).

In Experiment 2, mean gonadotropin concentrations were determined over 10-day intervals and then subjected to analysis of variance as described above. When serum concentrations of LH and FSH were below the sensitivity of the assays, lowest detectable values were used for numeric analysis.

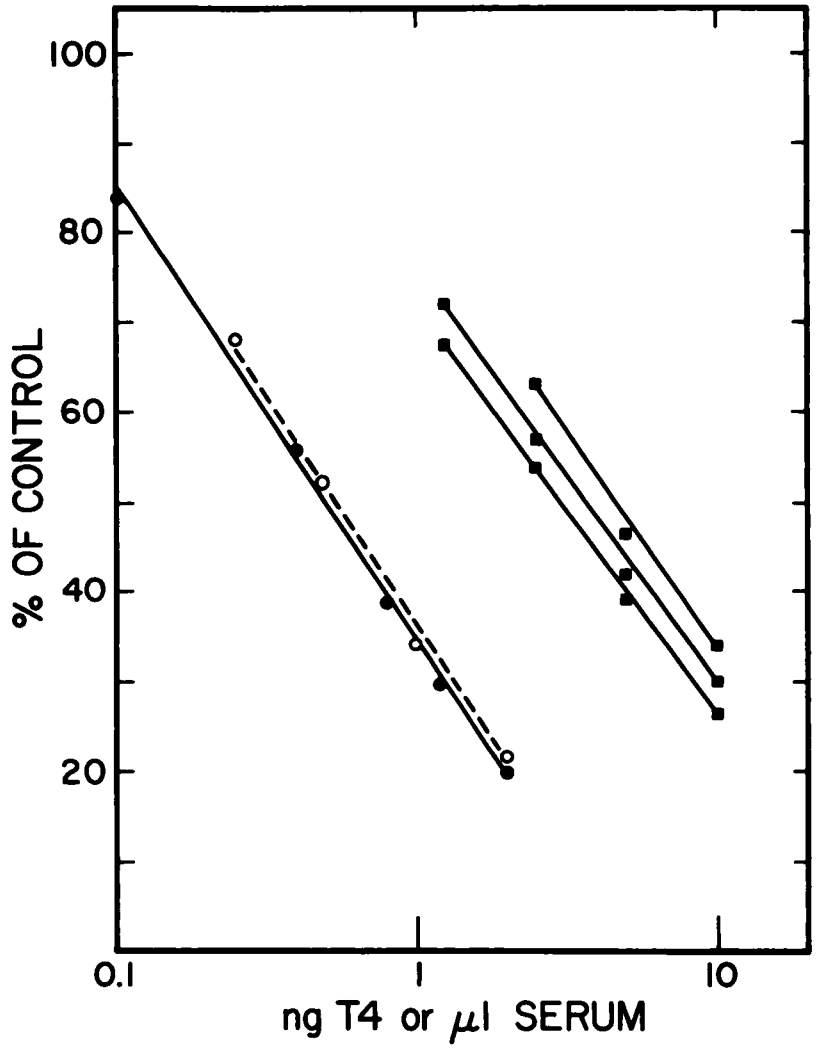

FIG. 1. Dose-response curves of the T_4 standard (Lot No. 7658, •——•), synthetic T_4 added to sera from thyroidectomized rhesus monkeys (o----o), and 3 serum pools from euthyroid rhesus monkeys (▪——▪) in the Gammacoat T_4 RIA.

RESULTS

Experiment 1

During the pretreatment control period, mean serum cortisol concentrations ranged from 17 to 35 μg/100 ml. Although treatment with the low dose of HCA did not result in a noticeable increment in circulating cortisol concentrations, institution of treatment with the high dose of HCA on Day 8 was followed by a slow and progressive rise in serum cortisol concentrations, which plateaued at approximately 150 μg/100 ml 32 days later. This elevation in circulating cortisol was sustained until approximately 1 wk after the withdrawal of HCA treatment, when serum cortisol concentrations began to decline, reaching pretreatment control levels 25 days later (Fig. 2). The elevations in serum cortisol concentrations during the last 7 wk of HCA treatment (Days 14–62) and during the first 2 wk of steroid withdrawal (Days 63–76) were statistically significant ($P<0.05$).

During the pretreatment control period, all four monkeys exhibited high circulating concentrations of LH and FSH. However, when serum cortisol had attained concentrations of 70–150 μg/100 ml, after approximately 20–30

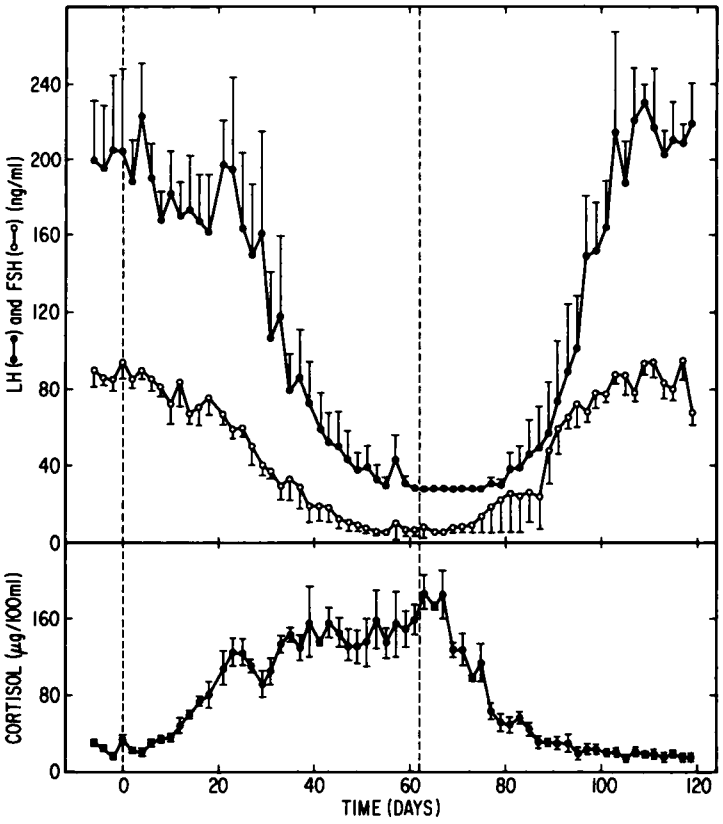

FIG. 2. Time courses of mean (± SEM) serum concentrations of LH (●——●) and FSH (○——○) in the *upper panel*, and cortisol concentrations in the *lower panel* in four castrated male rhesus monkeys during treatment with daily i.m. injections of HCA [10–20 mg/(kg BW·day)]. The duration of corticosteroid treatment is indicated by the *dotted vertical lines*. The elevation in cortisol between Days 14 and 76 was significant ($P<0.05$). Circulating LH and FSH concentrations were significantly ($P<0.05$) suppressed between Days 35 and 97 and Days 35 and 90, respectively.

days of HCA treatment, circulating LH concentrations began to progressively decline, reaching nondetectable values 12–30 days later (Fig. 2). Following the withdrawal of corticosteroid treatment, LH concentrations remained suppressed for 2–4 wk before a progressive rise in LH to precontrol levels was observed in association with the decline of serum cortisol concentrations to 25–35 μg/100 ml (Fig. 2). The depression in serum LH concentrations during the last 4 wk of HCA treatment (Days 35–62) and during the first 5 wk of steroid withdrawal (Days 63–97) was statistically significant ($P<0.05$). The time course of serum FSH levels during the experiment was similar to that of LH. Mean serum FSH concentrations during the last 4 wk of HCA treatment (Days 35–62) and during the first 4 wk of steroid withdrawal (Days 63–90) were significantly ($P<0.05$) lower than those at all other times.

Regression analysis demonstrated a statistically significant inverse correlation between circulating cortisol concentrations and LH ($r = -0.71$, $P<0.01$) and FSH ($r = -0.84$, $P<0.01$) levels. However, the foregoing correlations were most striking when the mean cortisol level during the n-th week of HCA treatment was compared with the mean concentrations of LH ($r = -0.96$) and FSH ($r = -0.95$) during the $n + 2$ week of treatment.

The serum T_4 concentration (mean ± SEM) during the last 5 days of HCA treatment was 13.2 ± 1.3 μg/100 ml, which compared to values of 11.6 ± 1.2 and 9.7 ± 1.6 μg/100 ml during the pre- and post-treatment control periods, respectively. Changes in body weight were not observed.

Experiment 2

As in the first experiment, the elevated serum cortisol concentrations that were pro-

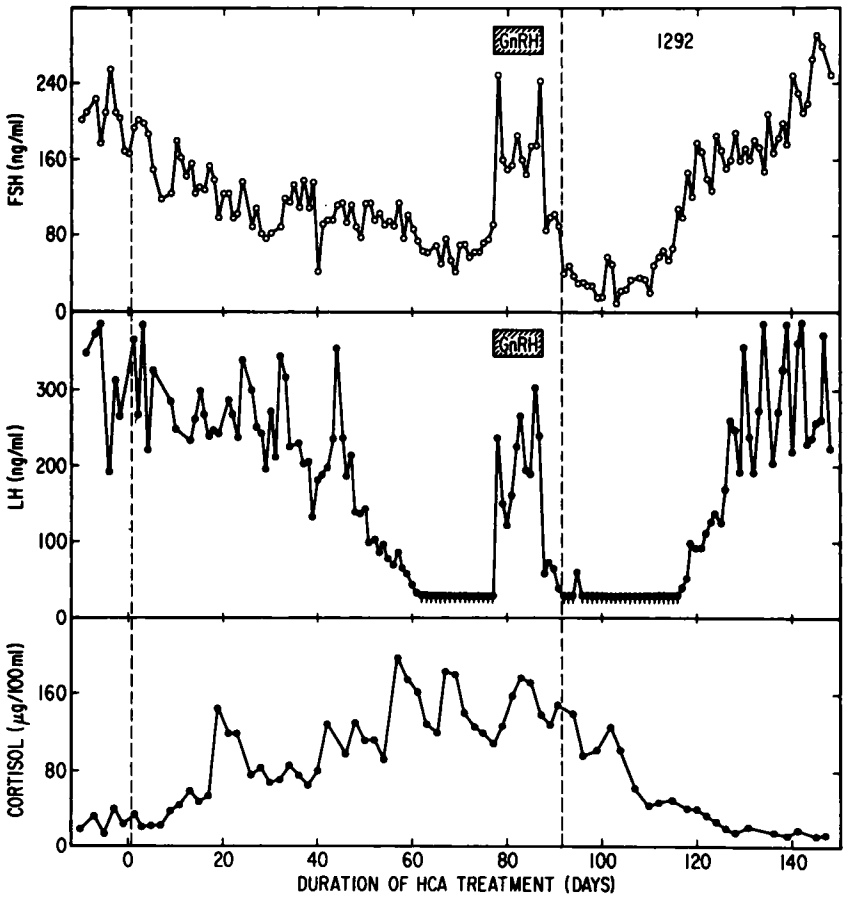

FIG. 3. Restoration of gonadotropin secretion by a chronic intermittent i.v. infusion of GnRH at 0.1 μg/min for 3 min every hour in a castrated male rhesus monkey, in which treatment with HCA at 20 mg/(kg BW·day) had suppressed circulating LH to nondetectable concentrations. An intermittent i.v. infusion of saline (1 ml for 3 min every hour) was administered for 4 days after termination of GnRH treatment. The sustained increment in serum cortisol levels produced by HCA treatment is shown in the *lower panel*. The *horizontal bar* and *vertical dotted lines* show the duration of GnRH and HCA treatment, respectively.

duced in monkeys 1290 and 1292 by the second period of HCA treatment resulted in a progressive decline in circulating LH and FSH concentrations. The initiation of an intermittent i.v. infusion of GnRH on Day 77 of HCA treatment when LH concentrations were no longer detectable elicited an immediate and significant ($P<0.05$) rise in serum gonadotropin concentrations in the face of the continued administration of HCA. In one of the animals, this GnRH-stimulated increase in gonadotropin secretion restored serum LH and FSH to concentrations that appeared indistinguishable from those observed during the pre- and post-treatment control periods (Fig. 3). In the second animal, the GnRH stimulation of gonadotropin secretion occurred more gradually, and by the eighth day of GnRH treatment, plasma LH and FSH concentrations had been restored to approximately 80% of pre- and post-treatment control levels (Fig. 4). Termination of the GnRH infusion 10 days later resulted in both animals in an immediate and significant ($P<0.05$) decline in serum LH and FSH to concentrations comparable to those observed prior to GnRH stimulation. When the HCA treatment was withdrawn, a progressive rise in serum LH and FSH to pretreatment control concentrations was again observed in association with a decline in circulating cortisol.

In agreement with the first experiment, serum T_4 concentrations were uninfluenced by

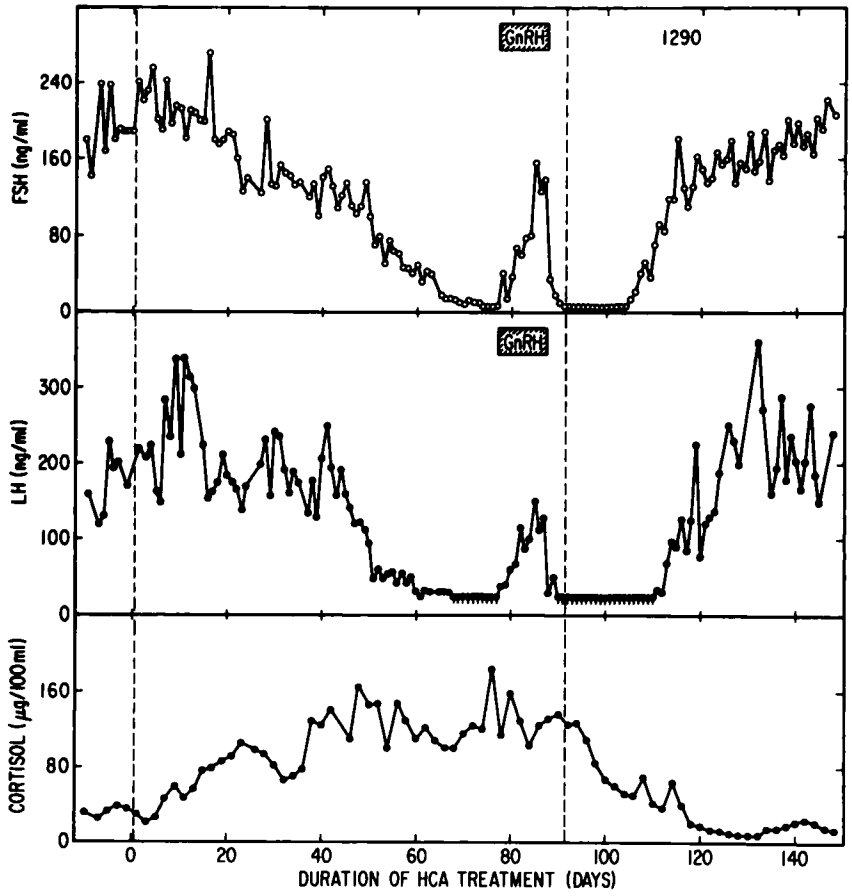

FIG. 4. A marked stimulation of LH and FSH secretion by pulsatile GnRH treatment in a castrated male rhesus monkey, in which treatment with HCA at 20 mg/(kg BW•day) had suppressed circulating gonadotropin to nondetectable concentrations. See also legend to Fig. 3.

HCA treatment. In contrast to the first experiment, the second period of corticosteroid treatment was associated with a 13% reduction in body weight.

DISCUSSION

The results of the present study confirm the earlier observation that a sustained elevation in plasma cortisol concentrations was associated with an inhibition of LH and FSH secretion in a castrated rhesus monkey (Plant and Zorub, 1984). While the suppression of open-loop gonadotropin secretion by corticosteroids does not seem to have been previously reported, the ability of these steroids to suppress the activity of adenohypophysial gonadotropes in intact animals is well established. In the bull, an acute elevation in circulating glucocorticoid levels produced either by administration of dexamethasone, a synthetic glucocorticoid, or by an injection of adrenocorticotropic hormone (ACTH) has been reported to inhibit LH and testosterone secretion (Chantaraprateep and Thibier, 1978; Johnson et al., 1982). A variety of glucocorticoids have also been reported to inhibit estrogen-induced preovulatory gonadotropin discharges and tonic LH secretion in rats and women (Baldwin and Sawyer, 1974; Cunningham et al., 1978; Baldwin, 1979). In addition, Cushing's syndrome in both men and women is characterized by hypogonadotropic hypogonadism (Boccuzzi et al., 1975; Luton et al., 1977).

Although it may be safely anticipated that ACTH release was compromised in the present study by HCA treatment (see Yates and Maran, 1974), the cortisol-induced inhibition of

gonadotropin secretion cannot be accounted for by a nonspecific, generalized suppression of adenohypophysial activity. Thyroid function, as reflected by circulating T_4 levels, and therefore presumably thyroid-stimulating hormone secretion, did not appear to be inhibited. Moreover, the deficit in gonadotropin secretion induced by sustained hypercortisolemia was restored by chronic intermittent stimulation with endogenous GnRH, a finding that suggests the ability of the gonadotropes to respond to an appropriate stimulus was not directly impaired by sustained exposure to elevated cortisol concentrations.

The foregoing notion that cortisol does not exert a major inhibitory action directly on the pituitary to blunt its responsivity to stimulation by GnRH is consistent with the preliminary finding that addition of cortisol or corticosterone to rat dispersed pituitary cells did not reduce LH secretion in response to GnRH stimulation (Suter and Schwartz, 1984). However, Li and Wagner (1983) and Padmanabhan et al. (1983), in similar studies of this problem, have reported that cortisol diminished the ability of dispersed pituitary cells to secrete LH in response to GnRH.

The intermittent i.v. infusion of GnRH employed in the present study (0.1 μg GnRH/min for 3 min once every hour) has previously been demonstrated to restore LH and FSH secretion in orchidectomized monkeys bearing hypothalamic lesions that abolish endogenous GnRH secretion (Plant and Dubey, 1984). It is reasonable, therefore, to conclude that this GnRH infusion provided the gonadotropes of the cortisol-treated monkeys with an exogenous stimulus similar to that provided by the central nervous system of untreated animals. Thus, the complete or near-complete restoration of the cortisol-induced suppression of LH and FSH secretion by intermittent GnRH stimulation provides compelling, albeit indirect, evidence for the view that the principal site of the inhibitory action of this glucocorticosteroid on gonadotropin secretion resided at a suprapituitary level and was mediated by an interruption of hypothalamic GnRH discharge. This notion is consonant with earlier observations that the application of glucocorticosteroids directly to the rat brain inhibited the pituitary-gonadal axis. Cortisol acetate implanted in the medial basal hypothalamus of immature male and female rats prevented the normal onset of puberty (Smith et al., 1971) and dexamethasone applied directly to the preoptic area in immature rats inhibited ovulation induced by the administration of pregnant mare's serum gonadotropin (Hagino et al., 1969).

The mechanisms that underlie the apparent arrest of intermittent GnRH secretion induced by chronic hypercortisolemia may only be speculated upon. Evidence from studies that have employed neurochemical and electrophysiologic approaches demonstrates that glucocorticoids may exert direct and specific actions upon neurons within the central nervous system. Glucocorticoid binding sites have been localized by autoradiography in hypothalamic and extrahypothalamic areas of the rat (Rees et al., 1975; Stumpf and Sar, 1976) and monkey brain (Pfaff et al., 1976). In the former species, a potent inhibition of electrical activity has been reported following the local application by iontophoresis of either dexamethasone or cortisol to hypothalamic neurons (Steiner et al., 1969; Mandelbrod et al., 1974). Furthermore, when glucocorticoids were added to incubates of hypothalamic fragments from rat brain, the production of corticotropin-releasing factor was immediately suppressed (Jones et al., 1976). Thus, it would seem reasonable to suggest that cortisol may be inhibiting GnRH secretion by a direct action on the neurons that synthesize this decapeptide.

The possibility remains, however, that the effects of sustained hypercortisolemia on hypothalamic GnRH secretion may be the consequence of an indirect and secondary, or even tertiary, effect of chronically elevated cortisol concentrations on metabolism and/or acid-base and electrolyte balance. In many mammalian species, high levels of cortisol produced either by treatment with ACTH or exogenous steroid result in marked perturbations in carbohydrate and protein metabolism (Steel, 1975), in metabolic alkalosis, and in hypokalemia (Grollman and Gamble, 1959). Comparable metabolic sequelae of hypercortisolemia have also been reported in various pathophysiologic conditions in man, such as Cushing's syndrome (Christy and Laragh, 1961; Travis, 1975; Johnston, et al., 1980). Although the effects of HCA treatment on metabolism were not systematically monitored in the present study, during the second experiment it was noted that animals were hypokalemic 4–5 wk after initiation of corticosteroid treatment. Thus, it seems reasonable to believe that many of the metabolic actions of excess

cortisol that have been documented extensively in studies by others were also in operation in the present series of experiments.

The physiologic significance, if any, of the finding that sustained hypercortisolemia inhibited open-loop gonadotropin secretion in the rhesus monkey remains to be established. Clearly, the circulating cortisol concentrations effected by HCA treatment were 4–8-fold greater than the basal levels of 10–30 μg/100 ml that were observed in the present study and that have been reported by others (Leshner et al., 1978; Plant, 1981). Although studies of the adrenal response to stress in this species are scant (Bajaj et al., 1979), we recently observed that imposition of restricted food intake for 3 wk, a physiologic perturbation, was associated with dramatic elevations in circulating cortisol levels that, in some animals, exceeded 80 μg/100 ml (Dubey et al., 1984), values within the range of those achieved in the present study by HCA treatment. In the context of the present discussion, it is also of interest to note that the hypercortisolemia induced by the foregoing nutritional insult, like that produced by exogenous steroid treatment in the present study, was associated with a GnRH-reversible inhibition of gonadotropin secretion (Dubey et al., 1984). Since the relationship between increasing serum cortisol concentrations and the suppression of gonadotropin secretion was characterized by an apparent hysteresis, it is unlikely that acute physiologic elevations in circulating cortisol markedly influence GnRH secretion. Nevertheless, it does not seem unreasonable to propose that an inhibition of GnRH secretion that appears to be occasioned by sustained elevations of serum cortisol may contribute to the physiologic mechanism that underlies impaired gonadal function in certain chronic "stressful" situations (Fries et al., 1974; Collu et al., 1979; Welsh and Johnson, 1981).

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