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Altered corticosterone status impairs steroidogenesis in the granulosa and thecal cells of Wistar rats

G. Valli, S. Sudha, B. Ravi Sankar, P. Govindarajulu, N. Srinivasan*

Department of Endocrinology, Dr. ALM PG Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai 600 113, India

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Abstract

Hypo- and hyper-corticosteronisms have adverse effects on ovarian endocrine and exocrine functions. In the present study, the mechanism by which corticosterone in excess or insufficiency impairs steroidogenesis in granulosa and thecal cells was investigated in adult albino Wistar rats. In this regard, rats were administered with corticosterone-21-acetate (2 mg/100 g b.wt., s.c., twice daily) or metyrapone (11β-hydroxylase blocker) (10 mg/100 g b.wt., s.c., twice daily) for 15 days and a group of corticosterone/metyrapone treated rats was withdrawn of treatment and maintained for another 15 days and killed during their diestrus phase. Administration of corticosterone-21-acetate while elevated the serum corticosterone levels, metyrapone diminished the same. Administration of metyrapone reduced the serum levels of LH and estradiol; corticosterone reduced the levels of FSH in addition to LH and estradiol. In vitro production of progesterone and estradiol by the granulosa and thecal cells was decreased due to altered corticosterone status. Whereas administration of corticosterone significantly reduced the activity of 3β-hydroxysteroid dehydrogenases (3β-HSD) in granulosa and thecal cells, it reduced the activity of 17β-HSD only in granulosa cells. While metyrapone treatment reduced the activity of 17β-HSD in granulosa as well as thecal cells, it reduced the activity of 3β-HSD only in thecal cells. The findings of the present investigation clearly demonstrate that excess or insufficiency in corticosterone affects steroidogenic process in the ovary. This is achieved by decreasing the levels of gonadotropins probably by their diminished synthesis and secretion and by interfering at the signal transduction process of these gonadotropins.

Keywords: Corticosterone; Metyrapone; Granulosa cells; Thecal cells; Steroidogenesis

1. Introduction

Evidences are accumulating to show that hypo- or hyper-corticosteronism impairs hypothalamo-hypophyseal-ovarian axis [1] and ovarian functions [2]. Identification of glucocorticoid receptors in the ovarian granulosa cells underlines the direct effects of glucocorticoid in the ovary [3]. In this regard, cortisol is reported to inhibit the stimulatory effects of FSH on aromatase activity in rat [4] and bovine granulosa cells in vitro [5]. It is also found to stimulate the specific activity of 3β-hydroxysteroid dehydrogenases (3β-HSD) in bovine granulosa cells [5]. Thus, glucocorticoids appear to interfere with the effects of gonadotropins in the ovary [6]. In the present study, the mechanism(s) by which altered corticosterone status impairs steroidogenic enzyme activities (3β- and 17β-HSD) was studied in ovarian cells of adult albino rats.

2. Materials and methods

2.1. Animals

Healthy adult female Wistar rats (75–80 days old) were maintained in a well ventilated and temperature controlled room with a 12 h light and 12 h dark schedule. The rats were fed with standard balanced rat pel-

* Corresponding author. Fax: +91-44-4926709.

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let (Lipton, India) and drinking water was made available ad libitum.

Rats showing regular estrous cycles were selected for the experiments and divided into five groups. Each group consisted of 15 animals.

Group I Control rats treated with equal volume (0.25 ml) of vehicle alone.
Group II Rats treated with corticosterone-21-acetate (2.0 mg/100 g b.wt., s.c., twice daily for 15 days).
Group III A group of corticosterone treated rats was withdrawn of treatment and maintained for another 15 days.
Group IV Rats treated with metyrapone (10 mg/100 g b.wt., s.c., twice daily for 15 days)
Group V A group of metyrapone treated rats was withdrawn of treatment and maintained for another 15 days.

Corticosterone-21-acetate and metyrapone were dissolved in 7% ethanolic propylene glycol and dimethyl sulfoxide, respectively. Metyrapone treated rats were given 0.9% sodium chloride along with drinking water to compensate the loss of mineralocorticoids and thereby the ionic loss. Since there were no significant differences in the mean values between 7% ethanolic propylene glycol and dimethylsulfoxide treated control rats as well as the control rats maintained for withdrawal groups, a common control was considered for statistical analysis of data.

Body weight and estrous cycles were monitored from the day of treatment until autopsy. At the end of experimental schedule, rats showing diestrus phase were killed by decapitation. Blood was collected, sera separated and stored at $-20^{\circ}C$ until used for the determination of serum profiles.

The ovaries were dissected out and adhering tissues and fat bodies were removed, blotted and weighed and processed for the isolation of granulosa and thecal cells. Granulosa and thecal cells were isolated and purified using Percoll gradient following the method of Magoffin and Erickson [7] and they were identified by histochemical staining for 3β-HSD [8]. Approximately 90% of the cells stained positive which reflected the purity of granulosa/thecal cell preparation. Viability of the cells was assessed by trypan blue exclusion test [8] and it was approximately 95%.

2.2. Enzyme assays

Purified granulosa/thecal cells (2×10^6) were used for the assay of 3β- and 17β-HSDs based on the method of Bergmeyer [9].

2.3. Quantification of serum hormones

Serum LH and FSH were estimated by radioimmuno assay using the antigens and antibodies supplied by NIADDK, USA. The cross-reactivity of LH with other hormones was $<0.02\%$ with rFSH, $<0.07\%$ and 0.01% with rPRL and that of FSH with other hormones was $<0.5\%$ with rLH and with rPRL $<0.01\%$. The sensitivity of LH assay was 0.14 ng/ml and of FSH was 0.2 ng/ml.

Serum progesterone and estradiol were assayed by RIA using kits obtained from Diagnostics Products (DPC), USA. The sensitivity of progesterone assay was 0.03 ng/ml and estradiol assay was 8 pg/ml. The cross-reactivity of progesterone antiserum to corticosterone, 17α-hydroxy progesterone and testosterone was 0.9%, 0.32% and non-detectable, respectively. The cross-reactivity of estradiol antiserum to estriol, estrone, progesterone and testosterone was 0.32%, 1%, non-detectable and 0.001%, respectively.

2.4. [^{125}I] LH/FSH binding studies

Binding studies were performed with iodinated (using lactoperoxidase) LH/FSH (NIADDK, USA) as described earlier [10].

Dispersed and purified granulosa/thecal cells were preincubated under 5% CO_2 and 95% O_2 at $37^{\circ}C$ for 12 h in 96 well plates at a concentration of 2×10^5 cells/0.3 ml culture medium (DMEM :Hams F12, containing 1% BSA with 1% FCS). The cells were then incubated with the FCS free medium in triplicate with [^{125} I] LH/FSH (10,000 cpm) and unlabeled hormone for 16 h at $4^{\circ}C$. Non-specific binding was determined with excess unlabeled hormone (1 μg). The final incubation volume was 0.3 ml. After the incubation period, the media were removed and wells were rinsed with 1× PBS twice. The cells were lysed with 0.1 N sodium hydroxide and the cell bound radioactivity was measured in an LKB gamma counter. Data were subjected to Scatchard plot analysis.

2.5. In vitro studies

2.5.1. Estimation of estradiol/progesterone levels in the conditioned media of ovarian cells

The ovaries were removed from corticosterone/metyrapone treated and the withdrawal group of rats; granulosa/thecal cells were isolated, suspended in DMEM containing 1% FCS, plated in 96 well plates (2×10^5 cells/well) and incubated separately under 5% CO_2 and 95% O_2 at $37^{\circ}C$ for 24 h. At the end of incubation, the medium was replaced with fresh medium without FCS and cultured for another 24 h. The granulosa cells were incubated with fresh medium containing FSH (100 ng/ml) and androstenedione (2 μM) for

48 h at 37°C. Thecal cells were incubated with LH (100 ng/ml) and pregnenolone (2 μM) for 48 h at 37°C. The culture media were collected at the end of incubation period, centrifuged and used for the assay of estradiol and progesterone by solid phase RIA method.

2.5.2. Statistical analysis

The data were analysed statistically by two way analysis of variance. Student's *t*-test was used to compare the degree of significance [11].

3. Results

3.1. Serum corticosterone

Administration of corticosterone raised (1.5 fold) and metyrapone decreased (five fold) the serum levels of corticosterone. Withdrawal of treatment almost brought back the levels to normal (Table 1).

3.2. Body weight, ovarian weight and estrous cycle

Administration of corticosterone resulted in a persistent diestrus stage, decreased the body ($p < 0.001$) and ovarian ($p < 0.01$) weights. Metyrapone treatment to rats prolonged the diestrus phase and registered a significant decrease ($p < 0.01$) in body weight. However, withdrawal of treatments restored these parameters to normal (Table 1).

3.3. Serum LH and FSH

Administration of corticosterone ($p < 0.01$) significantly decreased the serum levels of LH and FSH. Treatment with metyrapone also significantly reduced ($p < 0.01$) the serum levels of LH. Withdrawal of treatments brought back the levels to normal (Table 2).

3.4. [^{125}I] LH/FSH binding to ovarian cells

[^{125}I] LH/FSH binding to the granulosa cells of control rats was 5400 fmoles $\times 10^6$ cells and 2.845 fmoles $\times 10^6$ cells, respectively. The cells have single class of high affinity LH ($K_d \simeq 1.07 \times 10^{-11}$ M) and FSH ($K_d \simeq 1.29 \times 10^{-11}$ M) receptors. [^{125}I] LH binding to the thecal cells was 4.215 fmoles $\times 10^6$ cells and they had a single class of high affinity LH receptors ($K_d \simeq 1.25 \times 10^{-11}$ M). Neither the treatment (corticosterone or metyrapone) nor the withdrawal of treatments altered the binding capacity of [^{125}I] LH/FSH to the cells (data not shown).

3.5. Serum progesterone and estradiol

While treatment with metyrapone registered a significant increase ($p < 0.05$) in the levels of serum progesterone, withdrawal of treatment returned the levels to normal. However, administration of corticosterone had no remarkable effect. Administration of either corticosterone ($p < 0.01$) or metyrapone ($p < 0.05$) significantly reduced the levels of serum estradiol. Withdrawal of treatments brought back the levels of estradiol to normal (Table 2).

3.6. In vitro effects of FSH on progesterone and estradiol secretion by granulosa cells

The basal secretion of progesterone and estradiol by granulosa cells of corticosterone ($p < 0.01$) or metyrapone treated rats ($p < 0.05$; $p < 0.01$) was significantly diminished when compared to granulosa cells derived from controls (Table 3).

Granulosa cells collected from ovaries of control rats responded well to the FSH challenge in culture by secreting more progesterone and estradiol into the medium. However, granulosa cells derived from ovaries of corticosterone ($p < 0.001$; $p < 0.001$) or metyrapone ($p < 0.01$; $p < 0.001$) treated rats responded poorly with a diminution in progesterone and estradiol secretion (Table 3). In treatment withdrawal groups, the response was similar to that of controls.

3.7. In vitro effects of LH on progesterone secretion by thecal cells

The basal secretion of progesterone by thecal cells

Table 1
Effects of altered serum corticosterone levels on serum corticosterone, body weight and organ weight[a]

Experimental groups	Serum corticosterone (μg/dl)	Body weight (g)	Organ weight (mg/100 g b.wt.)
Control	38.0 ± 1.87	149.0 ± 3.00	31.33 ± 0.72
Corticosterone 21-acetate treated	$58.46 \pm 1.70^{***}$	$128.0 \pm 3.00^{***}$	$26.54 \pm 1.32^{**}$
Corticosterone withdrawal	40.35 ± 1.61	147.0 ± 2.00	29.08 ± 1.65
Metyrapone treated	$7.61 \pm 1.65^{***}$	$139.0 \pm 2.00^{**}$	28.53 ± 1.43
Metyrapone withdrawal	32.15 ± 2.72	147.0 ± 3.00	31.45 ± 1.53

[a] Each value is mean ± SEM of 10 observations. $^{***}p < 0.001$; $^{**}p < 0.01$ compared with control.

Table 2
Effects of altered serum corticosterone levels on serum concentrations of LH, FSH, progesterone and estradiol[a]

Experimental groups	LH (ng/ml)	FSH (ng/ml)	Progesterone (ng/ml)	Estradiol (pg/ml)
Control	6.79 ± 0.98	26.79 ± 2.51	8.11 ± 0.98	37.00 ± 5.23
Corticosterone treated	$2.08 \pm 0.17^{**}$	$15.07 \pm 1.92^{**}$	11.85 ± 2.13	$17.68 \pm 1.35^{**}$
Corticosterone withdrawal	5.85 ± 0.41	23.19 ± 2.24	9.70 ± 0.95	23.10 ± 3.02
Metyrapone treated	$3.84 \pm 0.29^{*}$	20.52 ± 2.41	$16.92 \pm 3.46^{*}$	$22.28 \pm 2.21^{*}$
Metyrapone withdrawal	5.78 ± 0.35	25.02 ± 1.91	9.91 ± 1.10	31.40 ± 3.84

[a] Each value is mean ± SEM of five estimations. $^{*}p < 0.05$; $^{**}p < 0.01$ compared with control.

derived from corticosterone ($p < 0.001$) or metyrapone treated ($p < 0.001$) rats was significantly decreased when compared to the basal production of progesterone in cells from control rats (Table 3).

While thecal cells collected from ovaries of control rats responded well to LH challenge in vitro by secreting more progesterone into the culture medium, thecal cells derived from ovaries of corticosterone ($p < 0.001$) or metyrapone ($p < 0.001$) treated rats responded with a decline in the secretion of progesterone (Table 3). In treatment withdrawal groups, the response was similar to that of control rats.

3.8. Steroidogenic enzymes

Administration of corticosterone significantly decreased the specific activities of 3β-HSD ($p < 0.05$) and 17β-HSD ($p < 0.01$) in granulosa cells. Whereas metyrapone treatment significantly decreased the activity of 17β-HSD ($p < 0.05$) only in the granulosa cells (Table 4).

Administration of either corticosterone or metyrapone significantly decreased ($p < 0.01$) the activity of 3β-HSD in thecal cells. However, metyrapone alone decreased the specific activity of 17β-HSD in thecal cells (Table 4). Withdrawal of treatments almost brought back the activities of these enzymes to normal in both the cell types.

4. Discussion

A number of experimental and clinical studies have underlined the positive and negative effects of altered serum glucocorticoids on hypothalamo-hypophyseal-ovarian axis in general and ovary in particular [1,2]. In the present study, the catabolic effects of glucocorticoids reflected well in rats given exogenous corticosterone and are consistent with earlier observations [12]. These effects may be direct or indirect through suppressing the production or actions of anabolic agents [13].

Metyrapone treated rats also showed a decrease in body weight albeit comparatively less than corticosterone treated rats. This may be due to deprivation of physiological levels of corticosterone and its implications on normal anabolic activities of the body.

Excess corticosterone is reported to affect the secretion of gonadotropins by interfering at the levels of hypothalamus and anterior pituitary gland in female rats [14]. The observed decrease in the levels of serum LH and FSH in rats treated with corticosterone may be due to a decrease in the synthesis and secretion of

Table 3
In vitro effects of FSH on progesterone and estradiol secretion in granulosa cells and LH on progesterone secretion in thecal cells obtained from corticosterone/metyrapone treated rats[a]

Experimental groups	Granulosa cells[b]				Thecal cells[b]	
	Progesterone[c]		Estradiol[c]		Progesterone[c]	
	Basal	Stimulated	Basal	Stimulated	Basal	Stimulated
Control	0.93 ± 0.08	3.20 ± 0.24	1.19 ± 0.16	3.49 ± 0.36	0.30 ± 0.02	0.83 ± 0.06
Corticosterone treated	$0.41 \pm 0.06^{**}$	$0.99 \pm 0.06^{***}$	$0.46 \pm 0.02^{**}$	$0.78 \pm 0.25^{***}$	$0.16 \pm 0.01^{***}$	$0.26 \pm 0.03^{***}$
Corticosterone withdrawal	0.86 ± 0.12	2.57 ± 0.09	0.90 ± 0.08	3.15 ± 0.37	0.26 ± 0.02	0.65 ± 0.07
Metyrapone treated	$0.69 \pm 0.05^{*}$	$1.94 \pm 0.11^{**}$	$0.64 \pm 0.06^{**}$	$0.95 \pm 0.31^{***}$	$0.18 \pm 0.01^{***}$	$0.28 \pm 0.01^{***}$
Metyrapone withdrawal	0.84 ± 0.13	2.82 ± 0.14	0.89 ± 0.06	3.57 ± 0.38	0.29 ± 0.02	0.72 ± 0.11

[a] Each value mean ± SEM of five estimations. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compare with control.
[b] Granulosa cells are stimulated with FSH (100 ng/ml); thecal cells LH (100 ng/ml).
[c] Progesterone values are expressed as ng/10^5 cells, Estradiol as pg/10^5 cells.

gonadotropins or GnRH or interference at the level of GnRH action on the gonadotropes by excess corticosterone.

Interestingly, a deficiency in corticosterone due to pharmacological adrenalectomy (metyrapone treatment) is also found to decrease the serum levels of LH. Probably an increase in serum progesterone in this group of rats has suppressed the secretion of LH. In addition, the elevated levels of ACTH in metyrapone treated rats might have reduced the secretion of LH as in the case of male rats [15].

The decrease in ovarian weight is comparable to a reduction in the levels of LH and FSH in corticosterone treated rats. This finding is also consistent with earlier reports wherein systemic dexamethasone treatment was found to decrease the ovarian weight [16]. In metyrapone treated rats, the unaltered ovarian weight is in correlation with the normal levels of FSH, suggesting a prime role for FSH in this regard.

The persistent diestrus after corticosterone and extended diestrus phase after metyrapone treatments point to derangements in the function of primary as well as accessory sex organs in these groups of rats as reported previously [17,18]. The decrease in serum levels of estradiol and normal to an increase in progesterone levels suggest derangements in reproductive functions.

It is well known that ovarian steroidogenesis is strictly under the regulation of gonadotropins [19]. Because of decrease in the secretion of gonadotropins, it is natural to expect a diminution in the ovarian steroidogenic process in hypo- or hyper-corticosteroid rats. This is evident from the decrease in specific activities of 3β- and 17β-HSD in granulosa as well as thecal cells and a significant decrease in the serum levels of estradiol. These findings are in agreement with earlier reports wherein LH and FSH are reported to stimulate the activities of these enzymes [20].

However, the normal levels of serum progesterone observed in corticosterone treated rats is more likely due to a decrease in the degradation rather than an increase in the synthesis of progesterone. In this regard, it is reasonable to point out that glucocorticoids are reported to decrease the activities as well as expression of 20α-HSD, a progesterone metabolising enzyme [21,22]. On the other hand, in the metyrapone treated rats, an increase in ACTH might have contributed for the increased output of progesterone from adrenal as reported earlier [14,15].

The in vivo studies have clearly demonstrated that an excess or deficiency in the levels of corticosterone affects ovarian steroidogenesis. A decrease in estradiol in the conditioned media of granulosa cells derived from corticosterone or metyrapone treated rats is consistent with the results obtained in vivo. Nevertheless, a significant decrease in the levels of progesterone in the conditioned media of granulosa cells derived from corticosterone as well as metyrapone treated rats, is contrary to the results obtained in vivo. Despite the provision of sufficient dose of LH or FSH, the decrease in the secretion of estradiol as well as progesterone by granulosa cells in the conditioned media indicates a defect (*s*) at the post-receptor signal transduction events. This postulation stems from the results on binding sites for LH and FSH in granulosa and thecal cells as there are no significant alterations in the number of binding sites for these hormones either after corticosterone or metyrapone treatment.

In this regard, it is pertinent to point out that dexamethasone is reported to act at the post cAMP level to inhibit aromatase activity in granulosa cells of rat ovary [23]. In addition to this, dexamethasone is known to inhibit the expression of $P_{450}scc$ gene in mouse Leydig cells [24] and cAMP induced promoter activity of the gene encoding $P_{450}scc$ and $P_{450}17\alpha$ hydroxylase in bovine adrenocortical cells [25]. Probably, in the current study also excess corticosterone might have interfered with cAMP mediated gonadotropin actions.

Our findings also reveal that the effects of corticosterone (excess or insufficiency) in diminishing the ster-

Table 4
Effects of altered serum corticosterone levels on 3β- and 17β-hydroxysteroid dehydrogenase activities in granulosa and thecal cells[a]

Experimental groups	3β-HSD[b]		17β-HSD[b]	
	Granulosa	Theca	Granulosa	Theca
Control	1.71 ± 0.24	3.82 ± 0.42	1.87 ± 0.22	0.99 ± 0.11
Corticosterone 21-acetate treated	$1.05 \pm 0.08^{*}$	$2.05 \pm 0.21^{**}$	$0.87 \pm 0.12^{**}$	0.82 ± 0.10
Corticosterone withdrawal	1.49 ± 0.22	3.37 ± 0.26	1.68 ± 0.26	0.89 ± 0.11
Metyrapone treated	1.45 ± 0.16	$1.97 \pm 0.21^{**}$	$1.14 \pm 0.14^{*}$	$0.59 \pm 0.09^{*}$
Metyrapone withdrawal	1.49 ± 0.18	3.46 ± 0.37	1.52 ± 0.30	0.92 ± 0.10

[a] Each value mean ± SEM of five estimations. $^{*}p < 0.05$; $^{**}p < 0.01$ compared with control.
[b] Values of 3β- and 17β-HSD were expressed as nmole of NADH formed/min/mg protein and nmole of NADPH formed/min/mg protein, respectively.

oidogenic activity in granulosa as well as in thecal cells are more or less on similar lines.

In conclusion, the present investigation clearly demonstrates that either an excess or deficiency in corticosterone affects the steroidogenic process in granulosa and thecal cells of adult rats by decreasing serum levels of gonadotropins and in addition probably by interfering in the signal transduction pathways of gonadotropins.

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