Metyrapone-Induced Corticosterone Deficiency Impairs Glucose Oxidation and Steroidogenesis in Leydig Cells of Adult Albino Rats

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Abstract. The present study was designed to identify the effects of metyrapone-induced corticosterone deficiency on Leydig cell steroidogenesis in adult male rats. Adult Wistar rats (200–250 g body weight) were treated with metyrapone, an inhibitor of corticosterone synthesis (10 mg/100 g body weight, s.c., twice daily) for 10 days. Experimental animals were killed along with controls, blood was collected, and sera separated for testosterone and estradiol assays. Testes were removed and Leydig cells were isolated, purified and used for estimating the specific activity of 17β-hydroxysteroid dehydrogenase (17β-HSD) and 14C-glucose oxidation. Serum testosterone (p<0.05), Leydig cellular 14C-glucose oxidation (p<0.001) and the specific activity of 17β-HSD (p<0.01) were significantly decreased in metyrapone treated rats. However, serum estradiol was not markedly altered compared to control. In addition to this, a set of in vitro experiments were also performed to identify the effects of metyrapone-induced corticosterone deficiency on hCG and prolactin-induced Leydig cell testosterone production. Metyrapone treatment significantly (p<0.05) decreased the Leydig cellular basal as well as hCG and its combination with prolactin stimulated testosterone production in vitro. It is concluded from the present study that the inhibitory effects of metyrapone-induced corticosterone deficiency on Leydig cell steroidogenesis are mediated through impaired glucose oxidation and 17β-HSD activity. In vitro studies showed that corticosterone deficiencies not only hCG action but also the potentiating effect of prolactin on Leydig cell steroidogenesis.

Key words: Leydig cell, Metyrapone, 17β-HSD, 14C-glucose oxidation, Testosterone

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STUDIES show that elevated endogenous glucocorticoids as a result of Cushing’s syndrome/disease and stress or supraphysiological doses of glucocorticoids decrease the plasma testosterone [1-5]. The direct effects of glucocorticoids on Leydig cell steroidogenesis have also been documented [6, 7]. Excess glucocorticoid impairs LH signal transduction and thus steroidogenesis in Leydig cells of adult rats [8, 9]. These effects of glucocorticoids on Leydig cells are receptor mediated [10, 11]. The metabolizing enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD), predominantly oxidative activity in adults, inactivates glucocorticoids present in Leydig cells [12]. Adrenal insufficiency as a result of Addison’s syndrome/disease, congenital adrenal hyperplasia due to 21-hydroxylase deficiency, X-linked adrenal hypoplasia congenita (AHC), adrenoleukodystrophy or adrenomyeloneuropathy and adrenoleukodystrophy also lead to hypogonadism [13-18]. Metyrapone, a specific adrenal 11β-hydroxylase inhibitor, specifically inhibits glucocorticoid production and also impairs the development of reproductive organs of prepubertal mice [19, 20]. Administration of metyrapone in-
hibits the spermatogenesis in langurs (Presbytis entellus entellus dufresnei) and testicular 17α-hydroxylation and side chain cleavage of 17α-hydroxylated C21 steroids in rats [21, 22]. Although a number of studies explain the effects of hypercortisolism or excess glucocorticoids on Leydig cell steroidogenesis, the specific effect of glucocorticoid deficiency on Leydig cell function is largely unknown. Therefore, the present study was designed to assess the effect of metyrapone-induced corticosterone deficiency on Leydig cell steroidogenesis in adult male rats.

**Materials and Methods**

**Animals**

Adult male Wistar rats (200–250 g) were divided into two groups. Group I-Control: Rats treated with equal volume of vehicle. Group II-Metyrapone treated: Rats treated with metyrapone (10 mg/100 g body weight, s.c., twice daily at 0900 h and 1800 h for 10 days). At the end of the treatment period, rats were killed by decapitation, trunk blood was collected and sera separated. Testes were removed and used for the isolation of Leydig cells.

**Serum hormone assays**

Serum testosterone was assayed using solid-phase RIA kit obtained from Diagnostic Products Corporation (DPC), USA. The sensitivity of the assay was 4 ng/dl, the intra- and interassay coefficient of variations were 4–11% and 7.3–11%, respectively. The cross-reactivity of the testosterone antiserum with estradiol was 0.02%. Estradiol was also assayed by solid-phase RIA kit obtained from DPC, USA. The intra- and interassay coefficient of variations were 4–7% and 4–8%, respectively and the cross-reactivity of the estradiol antiserum with testosterone was 0.001%.

**Leydig cell separation**

Testes were decapsulated under aseptic conditions and Leydig cells were isolated by enzymatic digestion and purified on discontinuous percoll gradient. The purity of Leydig cells was assessed by histochemical staining for 3β-HSD activity and viability was deter-

**17β-HSD enzyme activity**

The activity of 17β-HSD in Leydig cells was determined by the method of Bergmeyer [24]. Leydig cells (2 × 10⁶) were homogenized and sonicated in ice-cold tris-HCl buffer (pH 7.2) and centrifuged at 10,000 × g for 15 min at 4°C. The supernatant was then used as enzyme extract for the assay of 17β-HSD. The reaction mixture contained 0.6 ml of pyrophosphate buffer (100 μM), 0.2 ml of NADPH (0.5 μM), 2 ml distilled water and 0.1 ml of 4-androstene-3,17-dione (0.8 μM). After the addition of enzyme extract, the absorbance at 340 nm was measured at 20 sec intervals for 5 min in a spectrophotometer against blank. For total protein measurement, 0.02 ml of Leydig cell extract was taken and determined by the method of Lowry et al. [25] with bovine serum albumin as the standard. The specific activity of the enzyme was calculated and expressed as nanomoles of NADP formed/min/mg protein.

**Determination of 14C-glucose oxidation**

Leydig cell 14C-glucose oxidation was estimated by the method of Johnson and Turner [26] and Kraft and Johnson [27]. Twenty microliters of cell suspension containing 5 × 10⁶ cells were pipetted and placed in a 2 ml ampule containing 170 μl DMEM (pH 7.4), 10 IU penicillin in 10 μl of DMEM and 0.5 μCi of glucose. After aeration with gas mixture (5% CO₂ & 95% air) for 30 sec, the ampule was tightly closed with rubber cork containing CO₂ trap and incubated at 37°C. The CO₂ traps were replaced every 2 h. 0.01 ml of 1 N H₂SO₄ was added to the ampule, upon removal of second trap, to halt further metabolism. The system was again closed for 1 h before the third and final trap was removed and all the CO₂ traps were placed in the scintillation vials containing 10 ml of scintillation fluid and the samples were counted in a Packard scintillation beta counter. Results are expressed as CPM of 14CO₂ released/5 × 10⁶ cells.

**In vitro studies**

Leydig cells were purified from metyrapone and vehicle treated rats, plated in culture plates using
DMEM/F-12 containing 1% FCS and incubated under 5% CO₂ at 34°C for 12 h and the medium was replaced with FCS-free fresh medium containing the maximal effective doses of hCG (100 ng/ml/12 h) and PRL (75 ng/ml/12 h) to assess the Leydig cells response to hCG/PRL and their combinations. The culture media were collected at the end of incubation period and centrifuged. The supernatants were used for testosterone assay by solid-phase RIA.

Statistics

Data are expressed as mean±SEM and are analyzed for statistical difference by Student’s t-test.

Results

Serum hormones

Serum testosterone was significantly (p<0.05) decreased in rats treated with metyrapone (2.88 ± 0.44 ng/ml) compared to control (4.68 ± 0.39 ng/ml), but there was no significant change in serum estradiol level between control (4.3 ± 1.5 pg/ml) and metyrapone treated rats (6.1 ± 1.53 pg/ml) (Fig. 1).

17β-HSD enzyme activity

The activity of 17β-HSD enzyme was decreased in Leydig cells of metyrapone treated rats (0.23 ± 0.01 nmoles of NADP formed/min/mg protein) compared to that of control (0.29 ± 0.01 nmoles of NADP formed/min/mg protein) (p<0.01) (Fig. 2).

14C-glucose oxidation

Metyrapone treatment significantly (p<0.001) decreased the Leydig cell 14C-glucose oxidation (418 ± 149 CPM of 14CO₂ released/5x10⁶ cells) compared to control (5150 ± 827 CPM of 14CO₂ released/5x10⁶ cells) (Fig. 2).

Effects of metyrapone on hCG and PRL stimulated testosterone production by Leydig cells in vitro.

Basal, hCG and hCG plus PRL stimulated testosterone production were decreased in Leydig cells of metyrapone treated rats (1.19 ± 0.01, 1.99 ± 0.05, 4.21 ± 0.14 ng/10⁶ cells) compared to control (1.25 ±

![Fig. 1](image)

**Fig. 1** Effects of metyrapone on serum testosterone and estradiol.

- □ Control
- ■ Metyrapone treated

Each bar represents mean ± SEM of 5 animals. Significance at p<0.05; a-compared with control.
Fig. 2. Effects of metyrapone treatment on Leydig cell 17β-HSD activity and $^{14}$C-glucose oxidation.

- Control
- Metyrapone treated

Each bar represents mean ± SEM of 3 estimations, a & b represents statistical significance at p<0.01 and p<0.001, respectively compared with control.

Fig. 3. Effects of metyrapone treatment on hCG and hCG + prolactin stimulated testosterone production by Leydig cells.

- Control
- Metyrapone treated

Each bar represents mean ± SEM of 3 estimations; a-compared with respective control (p<0.05); b-compared with basal (p<0.01); c-compared with hCG treated (p<0.01).

0.01, 2.27 ± 0.04, 5.09 ± 0.12 ng/10⁶ cells) (p<0.05) (Fig. 3). However, a significant increase in testosterone production was recorded following hCG stimulation compared to basal in Leydig cells of both con-
trol and metyrapone treated rats (p<0.01). Combination of hCG and PRL also stimulated testosterone production significantly compared to hCG alone stimulated testosterone production in Leydig cells of both control and metyrapone treated rats (p<0.01).

Discussion

The results of the present study clearly demonstrate that metyrapone-induced glucocorticoid deficiency decreases testicular testosterone production. In accordance with the present study, others have also recorded similar changes in adrenalectomized or metyrapone treated rats [18, 22] and in patients with adrenal insufficiency due to congenital adrenal hyperplasia [28]. In addition to this, metyrapone treatment has been shown to produce a massive atrophy of the spermatogenic elements and shrinkage of seminiferous tubules and Leydig cells in langurs (Presbytis entellus entellus dufresne) [21]. Metyrapone inhibits formation of glucocorticoid, which induces a compensatory increase in CRH [29]. In vitro studies have shown that hCG-induced cAMP and testosterone production in rat Leydig cells are inhibited by CRH through its high affinity receptors in a dose dependent manner [30]. In addition to this, Andrea et al. [31] showed the production of CRF and its action via functional receptors to exert autocrine inhibitory action on Leydig cell steroidogenesis. It is therefore suggested that adrenal insufficiency associated decrease in serum testosterone may be due to the inhibitory effects of elevated CRF as a result of impaired negative feedback regulation caused by corticosterone deficiency.

Expression of testicular aromatase, which is involved in the synthesis of estrogens from androgens, is mainly restricted to Leydig cells of adult male rats [32]. In the present study, unlike serum testosterone, metyrapone treatment did not alter serum estradiol compared to control. However, metyrapone treatment significantly lowered 17β-HSD activity and testicular testosterone production, implying subnormal availability of testosterone and its subsequent transformation into estradiol. Therefore, it is proposed that the unaltered serum estradiol in metyrapone treated rats may be the result of impaired metabolic clearance of estradiol and/or increased peripheral conversion of androgens to estradiol.

The present study clearly demonstrates that the metyrapone-induced corticosterone deficiency inhibits Leydig cell 17β-HSD activity which is involved in the terminal step of testosterone synthesis [33]. Kwan and Gower [22] recorded that metyrapone can inhibit 17α-hydroxylase and side chain cleavage of 17α-hydroxylated C21 steroids in rat testicular microsomes in vitro. Pig testicular microsomal enzyme involved in the formation of C19 steroid from progesterone or pregnenolone was considerably inhibited by metyrapone [34]. In addition, in the female rats metyrapone treatment reduced the activity of 17β-HSD in granulosa and thecal cells [35]. Metyrapone specifically inhibited the enzymes 11β-hydroxylase and cytochrome P450sc in rat adrenal gland [36]. It is therefore suggested that the impaired activity of 17β-HSD in Leydig cells may be the direct effect of metyrapone. Since 17β-HSD activity is influenced by ATP and glucose availability [37], a decrease in Leydig cellular glucose oxidation recorded in the present study may also be responsible for the observed decrease in specific activity of 17β-HSD of metyrapone treated rats.

Leydig cells can utilize glucose predominantly and are suggested to be dependent on a continuous intake of energy-yielding substrates [38]. The present study clearly demonstrates that metyrapone-induced corticosterone deficiency decreased the Leydig cellular 14C-glucose oxidation. Khanum et al. [37] demonstrated that glucose availability plays an important role in the regulation of 17β-HSD activity in rat Leydig cells. Since glucose is required for Leydig cellular testosterone production [37, 39], the low level of testosterone in metyrapone treated rats may be the consequence of impaired glucose oxidation and the associated decrease in 17β-HSD activity. It has been shown that LH favours the glucose uptake by Leydig cells [39]. However, our previous study showed unaltered levels of serum LH in adrenalectomised male rats [40]. Therefore, it is proposed that the impaired glucose oxidation in the present study may be the consequence of metyrapone-induced corticosterone deficiency.

In vivo studies have clearly demonstrated that metyrapone-induced corticosterone deficiency affects testicular steroidogenesis. In order to assess the specific effects of hCG and PRL on testicular steroidogenesis under metyrapone-induced corticosterone deficiency, Leydig cells derived from control and
metyrapone treated rats were challenged with effective doses of hCG and hCG plus PRL in vitro for 12 h. LH is the principal trophic hormone regulating Leydig cell steroidogenesis [41]. Both LH and hCG bind to and activate the cell surface receptors [42]. Under normal condition, PRL maintains the action of LH on androgen biosynthesis and secretion in Leydig cells [43]. Johnson [44] reported that the treatment with LH plus PRL in rats resulted in greater stimulation of steroidogenesis than the animals treated with LH alone. In the present study, basal as well as hCG and hCG plus PRL-stimulated testosterone production in vitro were decreased in Leydig cells of metyrapone treated rats compared to control. Though hCG significantly increased the testosterone secretion in vitro by Leydig cells of both control and experimental rats compared to that of basal, the magnitude of increase was less in metyrapone treated rats, suggesting that the steroidogenic action of hCG is impaired due to metyrapone-induced corticosterone deficiency. Combination of hCG plus PRL significantly enhanced the testosterone production compared to that of basal as well as hCG alone stimulated testosterone production in Leydig cells of control rats. However, hCG plus PRL-induced increase in testosterone production by Leydig cells of metyrapone treated rats was not on a par with that of control implying their defective synergistic actions. Our previous study showed an increase in serum PRL due to adrenalectomy induced corticosterone deficiency [40]. Probably, an increase in PRL due to metyrapone-induced corticosterone deficiency might have caused down regulation of its own receptor and thus resulting in defective response to PRL.

It is concluded from the present study that metyrapone-induced corticosterone deficiency has inhibitory effects on Leydig cell steroidogenesis. The inhibitory effects of metyrapone-induced corticosterone deficiency are mediated through the suppression of 17β-HSD and glucose oxidation. In vitro studies indicate the defective steroidogenic effect of hCG and synergistic effects of hCG and PRL on Leydig cell testosterone production.

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