Endocrine Journal 2002, **49** (4), 405–412

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 ^{14}C-glucose oxidation. Serum testosterone ($p<0.05$), Leydig cellular ^{14}C-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 deficiency impairs not only hCG action but also the potentiating effect of prolactin on Leydig cell steroidogenesis.

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

(*Endocrine Journal* **49**: 405–412, 2002)

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 adrenalectomy 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-

Received: December 14, 2001
Accepted: March 26, 2002
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hibits the spermatogenesis in langurs (*Presbytis entellus entellus dufresne*) and testicular 17α-hydroxylation and side chain cleavage of 17α-hydroxylated C_{21} 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 determined by trypan blue exclusion test [23]. The purity of Leydig cells was 80–85% and viability was 90%.

17β-HSD enzyme activity

The activity of 17β-HSD in Leydig cells was determined by the method of Bergmeyer [24]. Leydig cells (2×10^6) 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 ^{14}C-glucose oxidation

Leydig cell ^{14}C-glucose oxidation was estimated by the method of Johnson and Turner [26] and Kraft and Johnson [27]. Twenty microliters of cell suspension containing 5×10^5 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% CO_2 & 95% air) for 30 sec, the ampule was tightly closed with rubber cork containing CO_2 trap and incubated at 37°C. The CO_2 traps were replaced every 2 h. 0.01 ml of 1 N H_2SO_4 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 CO_2 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 $^{14}CO_2$ released/5×10^5 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% CO_2 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).

^{14}C-glucose oxidation

Metyrapone treatment significantly ($p<0.001$) decreased the Leydig cell ^{14}C-glucose oxidation (418 ± 149 CPM of $^{14}CO_2$ released/5×10^5 cells) compared to control (5150 ± 827 CPM of $^{14}CO_2$ released/5×10^5 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/10^5 cells) compared to control ($1.25 \pm$

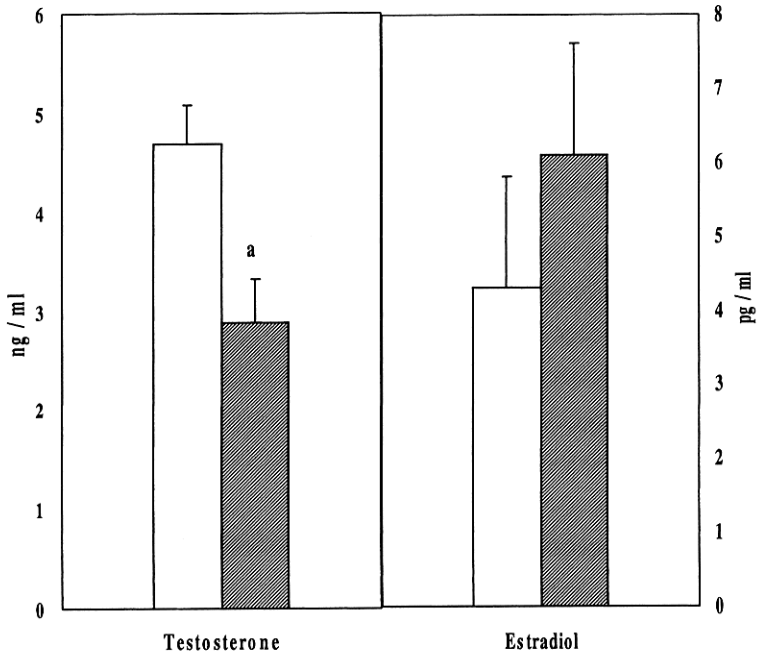

Fig. 1 Effects of metyrapone on serum testosterone and estradiol.
□ Control ▨ Metyrapone treated
Each bar represents mean ± SEM of 5 animals. Significance at $p<0.05$; a-compared with control.

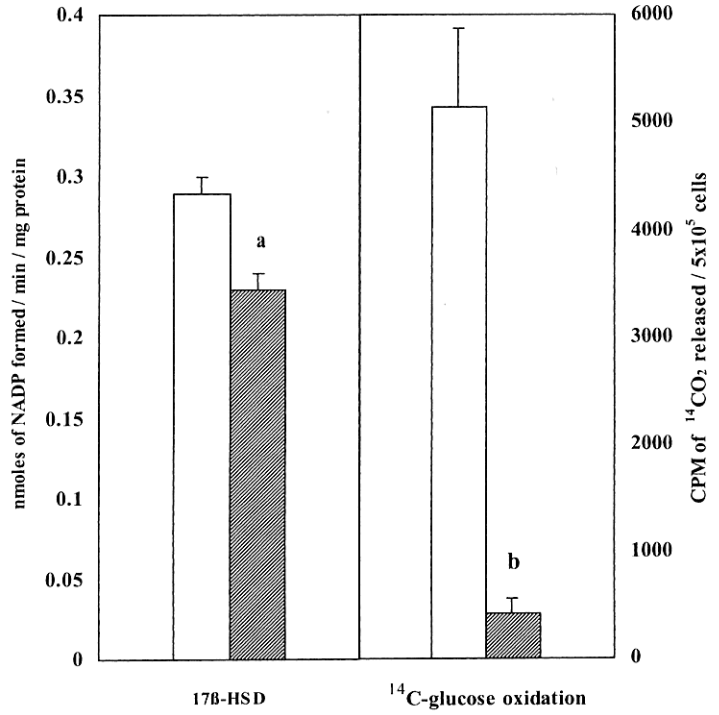

Fig. 2. Effects of metyrapone treatment on Leydig cell 17β-HSD activity and ^{14}C-glucose oxidation.
☐ Control ▨ Metyrapone treated
Each bar represents mean ± SEM of 3 estimations, a & b represents statistical significance at $p<0.01$ and $p<0.001$, respectively compared with control.

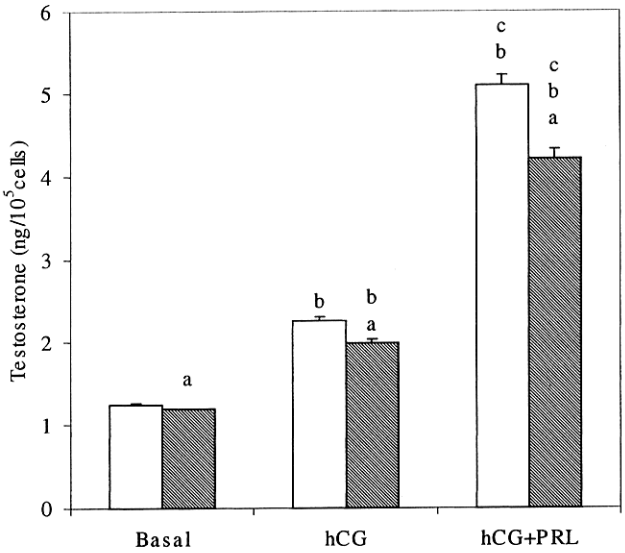

Fig. 3. Effects of metyrapone treatment on hCG and hCG + prolactin stimulated testosterone production by Leydig cells.
☐ Control ▨ Metyrapone treated
Each bar represents mean ± SEM of 3 estimations; a-compared with respective control ($p<0.05$); b-compared with basal ($p<0.01$); c-compared with hCG treated ($p<0.01$).

0.01, 2.27 ± 0.04, 5.09 ± 0.12 ng/10^5 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-hydroxylation and side chain cleavage of 17α-hydroxylated C_{21} steroids in rat testicular microsomes *in vitro*. Pig testicular microsomal enzyme involved in the formation of C_{19} 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 P_{450}scc 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 ^{14}C-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.

Acknowledgement

The authors wish to thank the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), USA, for providing hCG and prolactin as a gift and the UGC, New Delhi for the financial assistance through SAP-DRS programme.

References

1. Gabrilove JL, Nicolis GL, Sohval AR (1974) The testis in Cushing's syndrome. *J Urol* 112: 95–99.
2. Vierhapper H, Nowotay P, Waldhause W (2000) Production rates of testosterone in patients with Cushing's syndrome. *Metabolism* 49: 229–231.
3. Orr TE, Taylor MF, Bhattacharyya AK, Collins DC, Mann DR (1994) Acute immobilization stress disrupts testicular steroidogenesis in adult rat by inhibiting the activities of 17α hydroxylase and 17,20-lyase without affecting the binding of LH/hCG receptors. *J Androl* 15: 302–308.
4. Maric D, Kostic T, Kovacevic R (1996) Effects of acute and chronic immobilization stress on rat Leydig cell steroidogenesis. *J Steroid Biochem Mol Biol* 58: 351–355.
5. Veldhuis JD, Lizarralde G, Iranmanesh A (1992) Divergent effects of short term glucocorticoid excess on the gonadotropic and somatotropic axes in normal men. *J Clin Endocrinol Metab* 74: 96–102.
6. Payne AH, Sha LL (1991) Multiple mechanisms for regulation of 3β-hydroxysteroid dehydrogenase/Δ^{5-4}-isomerase, 17α-hydroxylase/C17–20 lyase cytochrome P450, and cholesterol side-chain cleavage cytochrome P450 messenger ribonucleic acid levels in primary cultures of mouse Leydig cells. *Endocrinology* 129: 1429–1435.
7. Hales LDB, Payne AH (1989) Glucocorticoid-mediated repression of P450scc mRNA and *de novo* synthesis in cultured Leydig cells. *Endocrinology* 124: 2099–2104.
8. Ravi Sankar B, Maran RRM, Sudha S, Valli G, Govindarajulu P, Balasubramanian K (1999) Corticosterone excess impairs LH signal transduction and steroidogenesis in Leydig cells of adult rat. *FASEB J* 13: 624.1.
9. Ravi Sankar B, Maran RRM, Sudha S, Govindarajulu P, Balasubramanian K (2000) Chronic corticosterone treatment impairs Leydig cell 11β-hydroxysteroid dehydrogenase activity and LH-stimulated testosterone production. *Horm Metab Res* 32: 142–146.
10. Evain D, Morera AM, Daez JM (1976) Glucocorti-

coid receptors in interstitial cells of the rat testis. *J Steroid Biochem* 7: 1135–1139.
11. Stalker A, Hermo L, Antakly T (1989) Covalent affinity labelling, radioautography and immunocytochemistry localize the glucocorticoid receptor in rat testicular Leydig cells. *Am J Anat* 186: 369–377.
12. Gao HB, Ge RS, Lakshmi V, Marandici A, Hardy MP (1997) Hormonal regulation of oxidative and reductive activities of 11β-hydroxysteroid dehydrogenase in rat Leydig cells. *Endocrinology* 138: 156–161.
13. Elder M, Maclaren N, Riley W (1981) Gonadal autoantibodies in patients with hypogonadism and/or Addison's disease. *J Clin Endocrinol Metab* 52: 1137–1142.
14. Valentino R, Savastano S, Tommaselli AP, Scarpitta MT, Dorato M, Gigante M, Calvanese E, Carlino M, Lombardi G (1997) Success of glucocorticoid replacement therapy on fertility in two adult males with 21-CAH homozygote classic form. *J Endocrinol Invest* 20: 690–694.
15. Peter M, Viemann M, Partsch CJ, Sippell WG (1998) Congenital adrenal hypoplasia: clinical spectrum, experience with hormonal diagnosis, and report on new point mutations of the DAX-1 gene. *J Clin Endocrinol Metab* 83: 2666–2674.
16. Seminara SB, Achermann JC, Genel M, Jameson JL, Crowly WF (1999) X-linked adrenal hypoplasia congenital: a mutation in DAX-1 expands the phenotypic spectrum in males and females. *J Clin Endocrinol Metab* 84: 4501–4509.
17. Brennemann W, Kohler W, Zierz S, Klingmuller D (1997) Testicular dysfunction in adrenomyeloneuropathy. *Eur J Endocrinol* 137: 34–39.
18. Lescoat G, Lescoay D, Garnier DH (1982) Influence of adrenalectomy on maturation of gonadotropin function in the male rat. *J Endocrinol* 95: 1–6.
19. Avgerinos PC, Cutler GB, Tsokos GC (1987) Dissociation between cortisol and adrenal androgen secretion in patients receiving alternate-day prednisone therapy. *J Clin Endocrinol Metab* 65: 24–29.
20. Pasley JN, McKinney RD, Blue LR (1975) Effects of metyrapone on reproductive organs of house mice. *Proc Soc Exp Biol Med* 148: 333–336.
21. Dixit VP (1978) Chemical sterilization of males—successful inhibition of spermatogenesis in langurs (Presbytis entellus entellus dufresne) after metopiron (Su-4885, Ciba) administration. *Endokrinologie* 72: 291–298.
22. Kwan TK, Gower DB (1988) Inhibition of rat testicular microsomal steroidogenesis by oxytocin and metyrapone. *Biochem Int* 16: 629–637.
23. Aldred LF, Cooke BA (1983) The effect of cell damage on the density and steroidogenic capacity of rat testis Leydig cells, using an NADH exclusive test for determination of viability. *J Steroid Biochem* 18: 411–414.
24. Bergmeyer HU (1974) Steroid dehydrogenases: In: Bergmeyer HU (ed) Methods of Enzymatic Analysis, Academic Press, New York, Vol. I: 476–477.
25. Lowry OH, Rosenbrough NJ, Lewis Farr A, Randall RJ (1951) Protein measurement with the folin phenol reagent. *J Biol Chem* 193: 265–275.
26. Johnson AD, Turner PC (1971) Epididymal carbohydrate metabolism I. Glucose 1-14C and glucose 614C metabolism by mouse, rat and rabbit tissues. *Comp Biochem Physiol* 39A: 599–604.
27. Kraft LA, Johnson AD (1972) Epididymal carbohydrate metabolism II. Substrates and pathway utilization of caput and cauda epididymal tissues from the rabbit, rat and mouse. *Comp Biochem Physiol* 42B: 451–461.
28. Combes-Moukhovsky ME, Kottler ML, Valensi P, Boudou P, Sibony M, Attali JR (1994) Gonadal and adrenal catheterization during adrenal suppression and gonadal stimulation in a patient with bilateral testicular tumors and congenital adrenal hyperplasia. *J Clin Endocrinol Metab* 79: 1390–1394.
29. Herman JP, Schafer MK, Thompson RC, Watson SJ (1992) Rapid regulation of corticotropin-releasing hormone gene transcription *in vivo*. *Mol Endocrinol* 6: 1061–1069.
30. Ulisse S, Fabbri A, Dufau ML (1989) Corticotropin releasing factor receptors and actions in the rat Leydig cell. *J Biol Chem* 264: 2156–2163.
31. Andrea F, Tinajero JC, Dufau ML (1990) Corticotropin-releasing factor is produced by rat Leydig cells and has a major local antireproductive role in the testis. *Endocrinology* 127: 1541–1543.
32. Carreau S, Bilinska B, Levallet J (1998) Male germ cells. A new source of estrogens in the mammalian testis. *Ann Endocrinol* 59: 79–92.
33. Chon HWA Tsai Morris, Azra Khanum, Pei-zhong Tang, Maria L Dufau (1999) The rat 17β-hydroxysteroid dehydrogenase type III: Molecular cloning of gonadotropin regulation. *Endocrinology* 140: 3534–3542.
34. Valli G, Sudha S, Ravi Sankar B, Govindarajulu P, Srinivasan N (2000) Altered corticosterone status impairs steroidogenesis in the granulosa and thecal cells of Wistar rats. *J Steroid Biochem Mol Biol* 73: 153–158.
35. Nakajin S, Takahashi M, Higashiyama K, Shinoda M (1985) Evidence for involvement of cytochrome P-450-linked oxygenase system in the conversion of C21-steroids to delta 16-C-19-steroids catalyzed by pig testicular microsomes. *J Biochem* 98: 615–620.
36. Williamson DG, O'Donnell VJ (1969) The interaction of metopirone with adrenal mitochondrial cytochrome P-450. A mechanism for the inhibition of

adrenal steroid 11β-hydroxylation. *Biochemistry* 8: 1306–1311.
37. Khanum A, Buczko E, Dufau ML (1997) Essential role of adenosine triphosphate in activation of 17β-hydroxysteroid dehydrogenase in the rat Leydig cell. *Endocrinology* 138: 1612–1620.
38. Hodgson Y, Hudson B (1983) Leydig cell function. *Monogr Endocrinol* 25: 107–132.
39. Amrolia P, Sullivan MH, Garside D, Baldwin SA, Cooke BA (1988) An investigation of glucose uptake in relation to steroidogenesis in rat testis and tumour Leydig cells. *Biochem J* 249: 925–928.
40. Balasubramanian K, Pereira BMJ, Govindarajulu P (1983) Effects of adrenalectomy and corticosterone replacement on epididymal carbohydrate metabolism. Studies on mature male rats. *Andrologia* 15: 71–77.
41. Hall PH (1994) Testicular steroid biosynthesis: organization and regulation. In: Knobil E and Neill JD (eds) The Physiology of Reproduction, Raven Press, New York, Vol. 1: 1335–1362.
42. Catt KJ, Dufau ML (1991) Gonadotropic hormones: biosynthesis, secretion, receptors and actions. In: Yen SCC and Jaffe RB (eds.) Reproductive Endocrinology, WB Saunders, Philadelphia, 105–155.
43. Bartke A (1976) Pituitary-testis relationships: role of prolactin in the regulation of testicular function. In: Hubinot PO (ed) Sperm Action, Basil, Karger, Vol. 1: 136–152.
44. Johnson DC (1974) Temporal augmentation of LH by prolactin in stimulation of androgen production by the testes of hypophysectomized male rats. *Proc Soc Exp Biol Med* 145: 610–613.