REVIEW

Glucocorticoids Affect Male Testicular Steroidogenesis

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Summary

Through their receptors at each level of hypothalamo-pituitary-gonadal axis glucocorticoid excess, either endogenous or administered or stress-induced, could affect steroid production in the testis and thus male fertility. The main ways by which glucocorticoids act are as follows: 1) Affecting gonadoliberin and LH synthesis and release through glucocorticoid receptors in hypothalamic neurons and pituitary gonadotropes. 2) By so far not clearly evidenced reduction of the number of LH receptors on the membrane of Leydig cells. 3) By affecting expression and function of steroidogenic enzymes in the testis. 4) By regulation of *in situ* access of glucocorticoid to its target cells in the testis. 5) By promotion Leydig cell apoptosis. The review provides a survey of physiological and molecular mechanisms staying behind these effects. It does not deal with the clinical effects of glucocorticoid treatment which would substantially exceed the scope of the pater.

Key words

Glucocorticoid • Stress • HPG axis • Leydig cell • Apoptosis

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Introduction

Clinical experience as well as many experimental studies brought evidence that excess of glucocorticoids (GCs), either administered or stress-induced, impacts testicular function and thus male fertility (Hu *et al.* 2008, Whirledge and Cidlowski 2010, Nargund 2015, Witorsch 2016). In this review we tried to survey multiple physiological and molecular mechanisms staying behind these effects.

As documented in many situations, stress inhibits reproductive functions. An unusual example could be shown here. During five days' military training course for Norwegian male cadets with hard physical activity day and night and almost no sleep or food, a decrease was found in LH, FSH, prolactin and thyreotropin. A decrease was also found in androgens testosterone, dihydrotestosterone (DHT), androstenedione, dehydroepiandrosterone and 17α-hydroxyprogesterone, whereas dehydroepiandrosterone-sulfate increased twofold. The LH and FSH responses to gonadoliberin (GnRH) intravenously were increased at the end of the course. This demonstrates enhanced pituitary reserves of gonadotropin, or, alternatively, increased sensitivity to GnRH stimulation and may be due to decreased hypothalamic secretion of GnRH during the course. The decreased DHT and testosterone levels were almost normalized after hCG stimulation, indicating a gonadotropin regulated decrease in testosterone secretion during the course. In spite of fairly weak correlation between the alteration in gonadotropins and androgens it is concluded that there is a major regulation of testicular androgen secretion during prolonged stress by the hypothalamo-pituitary axis. Psychogenic effect on the androgen levels could be demonstrated by the even more deep decease of androgens in a subgroup of cadets who were methodically under bullying of the officers (Opstadt 1992).

Many of these actions are mediated by glucocorticoid receptors (GR). Nuclear GR are present in almost all cells of the body, including receptors at each level of hypothalamo-pituitary-gonadal (HPG) axis. In addition, rapid non-genomic actions of GC should be

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considered (Dong *et al.* 2004). Most experimental data have been obtained on rodents, but the results are of importance also for human medicine since corticoids belong to mass used therapeutics and may affect considerably male fertility.

The main ways GCs utilize for affecting and regulation of testicular function may be summarized as follows: 1) By affecting gonadoliberin and LH synthesis and release through GR in hypothalamic neurons and pituitary lutropes. 2) By influencing the number of LH receptors on the membrane of Leydig cells through local GR. 3) By affecting expression and function of steroidogenic enzymes in the testis. 4) By regulation of *in situ* access of glucocorticoid to its target cells in the testis. 5) By promotion Leydig cell apoptosis.

Glucocorticoid effect on GnRH and LH synthesis and release

Testicular steroidogenesis is governed by hypothalamo-pituitary-gonadal axis (HPG), while glucocorticoid balance is maintained by hypothalamo-pituitary-adrenal (HPA) axis. In both instances the regulation takes advantage of negative as well as positive feed-back loops and thanks to receptors at each level, a cross talk exists between both axes. The signaling molecules of the HPG axis are able to modulate corticoid signaling and at the same time the hormones of the HPA axis, first of all corticosteroids, but also other stress hormones, influence the HPG axis (Oyola and Handa 2017, Acevedo-Rodriguez *et al.* 2018).

As early in seventies (Sakakura *et al.* 1975) demonstrated that glucocorticoid treatment of humans led to inhibition of luteinizing hormone secretion induced by synthetic gonadoliberin. Indirect evidence for GR-mediated inhibition of pituitary LH secretion by GC brought Briski *et al.* (1995) showing that GR antagonist RU486 attenuated the acute decline in circulating LH in stressed rats. Expression of GR was confirmed in mouse gonadotropes along with the finding that treatment with GC reduces GnRH-induced specific LHβ expression (Breen *et al.* 2012, Breen and Mellon 2014).

Recent discovery of hypothalamic kisspeptin and its partial antagonist gonadotropin-inhibitory hormone (GnIH)/RFamide-related peptide 3 (RFRP-3), revealed further common sites in both axes. These peptides differ in their hypothalamic localization and both regulate gonadoliberin secretion but in a different way: kisspeptin directly stimulates GnRH synthesis and release, while RFRP-3 acts in both hypothalamus and pituitary by suppressing the release and synthesis of GnRH and gonadotropins. Kisspeptin and RFRP-3 expression and activity are decreased and increased by stress, respectively, pointing to the role of stress induced hormones, first of all glucocorticoids. Indeed, corticoliberin (CRH), which is the driving factor of HPA axis is another potent inhibitor of GnRH secretion and on the hypothalamic neuron systems may influence kisspeptin signaling (Iwasa *et al*. 2018, Acevedo-Rodriguez *et al.* 2018).

The receptor for GnIH is the G protein-coupled receptor 147 (GPR147), which inhibits cAMP signaling. GnIH can inhibit gonadotropin synthesis and release by decreasing the activity of GnRH neurons as well as by directly inhibiting pituitary gonadotrope activity. GnIH and GPR147 can thus centrally suppress testosterone secretion and spermatogenesis by acting in the hypothalamic-pituitary-gonadal axis. GnIH and GPR147 are also expressed in the testis of birds and mammals, possibly acting in an autocrine/paracrine manner to suppress testosterone secretion and spermatogenesis. GnIH expression is also regulated by melatonin, stress, and social environment in birds and mammals (Ubuka *et al.* 2014)

Effect of glucocorticoids on lutropin receptors in Leydig cells

Stimulation of testicular steroidogenesis by lutropin is mediated by membrane G protein-coupled LH receptors (LHR). In an early study on hypophysectomized rats and testicular cell culture, treatment with dexamethasone or corticosterone led to decrease of testicular LH/hCG receptor content as measured by [^{125}I]hCG binding assay. Some animals were also given concomitantly FSH to prevent the hypophysectomy-induced decrease in testicular functions (Bambino and Hsueh 1981). The question arose later whether the effect was caused by GR-mediated suppression of LHR expression. The issue was partially addressed by Parthasarathy and Balasubramanian (2008), who measured mRNA of LHR and LH-binding in purified rat Leydig cells after metopyrone induced corticosterone deficiency and subsequent corticosterone replacement. In contrast to previous authors, they did not find significant changes in LH mRNA expression, but it should be stressed that they did not measured the single effect of GCs on LHR mRNA, but only the effect of

induced GC deficiency. Thus the question remains open and further studies are needed to confirm definitely if the expression of LHR is inhibited by corticoids.

Effect of glucocorticoids on testicular steroidogenesis

A number of studies dealt with various aspects of GC effect on Leydig cell steroidogenesis. A decrease of testosterone production after GC treatment was observed already by above mentioned authors (Bambino and Hsueh 1981). In their next work using primary cultures from rat testicular cells they compared the inhibitory effect of GC on other steroid precursors in testosterone biosynthesis, which declined in the order androstenedione (80 % decrease) to 17α-hydroxyprogesterone (57 %), with a lesser effect on progesterone production (28 % decrease) and no effect on pregnenolone production (Welsh *et al.* 1982). Later studies on rats definitely proved direct inhibitory effect of GC on expression on two enzymes of testosterone biosynthesis, namely 3β- and 17β-hydroxysteroid dehydrogenases at their mRNA level (Badrinarayanan *et al.* 2006).

The effect of glucocorticoid on enzymatic activity and expression of genes encoding for androgen biosynthetic enzymes was also studied in progenitor Leydig cells from 21-day-old rats, which differ from adult Leydig cells in many aspects. The activity of three mitochondrial enzymes of androgen biosynthesis, namely cholesterol side chain cleavage enzyme, 3β-hydroxysteroid dehydrogenase and 17α-hydroxylase/ 20-lyase plus one androgen metabolizing enzyme, 5α-reductase, were measured, along with their protein expression. For the first time they also measured expression of steroid acute regulatory protein (StAR), mediating cholesterol transport across the inner mitochondrial membrane. Generally, GCs decreased activity and expression of the enzymes, though in different way than in adult cells. The effects were inhibited by GR antagonist mifepristone (RU486) confirming GR mediation (Xiao *et al.* 2010).

Let us mention here a rapid decrease of testosterone production following immobilization stress of mice. In the experiment of Dong *et al*. (2004), no effect on LH levels was observed within 6 h after stress in contrast to rapid decrease of testosterone within 30 min, accompanied with a decrease of intracellular cAMP. On the other hand, the reduction of testosterone was reversed by GR antagonist RU486, suggesting a non-genomic mechanism *via* putative membrane located glucocorticoid receptors.

Regulation of an access of glucocorticoid to its target cells in the testis

Actual concentration of glucocorticoids and their access to their target tissue is controlled by activity of local microsomal enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD), catalyzing oxidoreduction (interconversion) of cortisol or corticosterone to their biologically inactive 11-oxo metabolites. The enzyme exists in two isoforms – 11β-HSD1 and 11β-HSD2. 11β-HSD1 functions in both directions (oxidation or reduction) using NADPH/NADP+ as cofactors, while 11β-HSD2 possess exclusively oxidative activity with NAD+ as a cofactor. 11β-HSD1 is ubiquitous, being abundant especially in liver, 11β-HSD2 occurs selectively in several tissues as in kidney and also in the testis (Quinkler *et al.* 2001, Sandeep and Walker 2001, Wang *et al.* 2019, Šimůnková *et al.* 2011, Ergang *et al.* 2014, Máčová *et al.* 2015). Both isoforms are thus present in the testicular Leydig cells but 11β-HSD2 contribution to regulate active corticoid access is much lower than that of 11β-HSD1 (Ge *et al*. 2005).

The studies with animals and tissue cultures showed that the mechanism used by 11β-HSD1 in the liver differs from that operating in Leydig cells. In the former its reductive activity is governed by actual availability of NADPH, generated by hexose-6-phosphate dehydrogenase with glucose-6-phosphate as a substrate. In Leydig cells 11β-HSD1 is coupled with another enzyme, 17β-hydroxysteroid dehydrogenase (17β-HSD) mentioned above. Both enzymes are localized in smooth endoplasmic reticulum membrane. The testosterone precursor androstenedione and NADPH are used by 17β-HSD1 to generate NADP+, enabling 11β-HSD1 to function as an oxidase by oxidation of corticosterone to inactive 11-dehydrocorticosterone (Hu *et al.* 2008).

Do GCs affect Leydig cells apoptosis?

With respect to well-known promotion of cell apoptosis by GCs and predominantly harmful effects of GCs on Leydig cells function it was of interest, whether the excessive glucocorticoid stimulation also affects total Leydig cell numbers through induction of apoptosis and if so, by which mechanism (Hardy *et al.* 2005). Studies

from the same author´s group using cultured Leydig cells from adrenalectomized rats proved an increase of Fas Ligand and Fas receptor protein, the key players in starting caspase signaling cascade, characteristic for inner apoptotic pathway, following corticosterone administration. Small DNA fragments typical for apoptosis were seen in Leydig cells as well and addition of a specific caspase inhibitor, Ac-DEVD-CHO led to suppression of apoptosis (Gao *et al.* 2003). Further studies *in vivo* using chronically stressed rat model brought clear evidence that GCs induced apoptosis proceeded *via* glucocorticoid receptors and thus a genomic mechanism takes place here (Chen *et al.* 2012). Blockade of intratesticular glucocorticoid receptors diminished proapoptotic action without affecting antiapoptotic effects (Andric *et al.* 2013).

Conclusions

In the review we have shown how glucocorticoids affect testicular steroidogenesis in the light of recent knowledge of the mechanisms operating at individual levels of hypothalamo-pituitary-gonadal axis. The main ways responsible for the final GC effect are the following: through their receptors in hypothalamus, gonadotropes and in the Leydig cells, GC affect gonadoliberin and LH synthesis and release, and the expression and function of steroidogenic enzymes. In addition, glucocorticoid access to its target cells in the testis is controlled by activity of local enzyme 11β-hydroxysteroid dehydrogenase. The other effects include so far not clearly evidenced reduction of the number of LH receptors on the membrane of Leydig cells and promotion of Leydig cell apoptosis. Most results have been obtained on animal models and some also by using tissue cultures, but they are valuable also for human medicine since glucocorticoids belong to mass-used therapeutics. We did not review here the effects of glucocorticoid treatment on spermatogenesis and sperm function, which however, is tightly bound to testicular steroidogenesis.

Conflict of Interest

There is no conflict of interest.

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Abbreviations

LH – luteinizing hormone, FSH – follicle stimulating hormone, hCG – human chorionic gonadotropin, NADPH – nicotinamide adenine dinucleotide phosphate.

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