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Androgen Dependency of the Pituitary-Adrenal Axis in the Gerbil *(Gerbillus tarabuli)* (Thomas, 1902) Living in the Algerian Sahara Desert

Dependencia Androgénica del Eje Hipofisiario-Suprarrenal en el Jerbo (*Gerbillus tarabuli*) (Thomas, 1902) que Habita en el Desierto del Sahara Argelino

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SUMMARY: We previously reported that the free-living male *Gerbillus tarabuli* exhibits opposite seasonal variations of the pituitary adrenal axis activity to that of the pituitary gonadal axis, suggesting negative interrelationships between these endocrine functions. So, in this paper we investigate the role of testosterone on pituitary adrenal axis by removing testes during the sexually active period. Gerbils were captured in late winter and early spring in their natural Saharan biotope. The experiment involved three groups of eight adult males (sham-operated control, gonadectomized and testosterone-replaced gonadectomized gerbils). The right adrenal and pituitary glands were quickly taken out from euthanasia performed 30 days after gonadectomy or 7 days after testosterone replacement. Then, they were fixed either for beta-catenin (β-catenin) immunohistochemistry in the adrenal glands using specific β-catenin antibody or double-labelled indirect immunofluorescence for androgen receptors (AR) and ACTH cell detection in the pituitary sections using a rabbit polyclonal anti-AR antibody and a mouse monoclonal antibody against ACTH. In the intact gerbils, AR were found colocalized with ACTH in pituitary corticotroph cells. The adrenocortical immunolocalization of β-catenin was particularly capsular and slightly glomerular, whereas it was absent in the inner zona cortical. After orchiectomy, ACTH cells were less numerous with a weak expression of AR. In the adrenal cortex, castration induced a strong β-catenin immunoreactivity in the fasciculata and zona reticularis with a centripetally decreasing gradient; b-catenin immunoreactivity disappears completely in the adrenal capsule. Testosterone replacement therapy restores all parameters. These results suggest an inhibitory effect of testosterone on the pituitary-adrenal axis of *Gerbillus tarabuli*; this steroid could act via a central pathway and locally with an important position of canonical Wnt/β-catenin signaling in order to preserve adrenal cortex zonation and homeostasis throughout the year in this seasonally breeding Saharan rodent.

KEY WORDS: Saharan gerbil; Adrenal homeostasis; ACTH cells; β-catenin; Androgen dependency.

INTRODUCTION

The adrenal glands produce various hormones that control many biological and biochemical factors. Based on different embryological origins, structural characteristics, and specific-released hormones, the adrenal glands comprise a mesenchymal capsule covering three cortical layers of specific cells surrounding the central medulla. The medullary catecholamines prepare the body against stress. The adrenal cortex produces several steroid hormones by specialized cells arranged in concentric layers (Miller & Auchus, 2011). Mineralocorticoids are secreted by the outermost zone, named zona glomerulosa (zG), while glucocorticosteroids, which regulate stress and immune responses, are produced in the middle zone, named zona fasciculata (zF) and adrenal androgens are released from the inner zone, named zona reticularis (zR). In addition, the adrenal capsular is considered as a signaling center that plays a pivotal role in cortical zonation and tissue renewal (Pignatti *et al*., 2020). In some species, such as the desert gerbil, a layer of connective tissue bands separates the adrenal cortex from the medulla (Zatra *et al*., 2018).

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In mammals, the mechanism behind the maintenance of adrenal cortex balance and renewal is still poorly understood. One of the adrenocortical stem cell types, Wilms Tumor 1 (WT1), makes a minimal contribution to the preservation of homeostasis in adulthood (Bandiera *et al*., 2013). However, it was reported that WT1-expressing cells migrate into the cortical layers in order to differentiate into SF1-expressing steroidogenic cells (Bandiera *et al*. 2013). Additionally, several elements of the Wnt signaling pathways in the mouse adrenal cortex have been found to have a previous place for Wnt/β-catenin, essentially in the zG (Kim *et al*., 2008). Other studies have demonstrated that roof plate-specific spondin (RSPO), which is secreted from the mesenchymal capsular cells, activates β-catenin in subcapsular steroidogenic cells, thereby determining the fate of the outermost cortical layer, the zG. Some authors have reported that zonation depends on the antagonism of β-catenin with PKA signalling (Drelon *et al*., 2016). Like the majority of hormones, testosterone acts on its target tissues through its specific Androgen Receptor (AR). Numerous studies have documented a broad distribution of AR in desert rodents (Benmouloud *et al*., 2014; Aknoun-Sail *et al*., 2017; Zatra *et al*., 2018) and rats (Trejter *et al*., 2015; Ajdzanovic *et al*., 2016) as well as in the reproductive organs and other peripheral tissues, including the adrenal glands.

In seasonally reproducing species, the reproductive cycle and adrenal activity suggest interrelationships that may link these functions in order to ensure both survival and sustainability in extreme Desert environments (Bauer *et al*., 2014). In most Saharan mammals, sexual activity is limited to the period of the year that allows birth at the most favorable time for offspring survival. This adaptation requires important metabolic adjustments involving the regulatory endocrine glands, particularly the adrenal cortex. Conversely, sex hormones play a role in the function of the adrenal cortex (Trejter *et al*., 2015). Recent papers have shown that many factors and hormones that allow cell remodeling in order to meet steroid needs (Pihlajoki *et al*., 2015; Zuloaga *et al*., 2024) can target the hypothalamic-pituitary axis. In the Saharan rodents, the involvement of testicular androgens in pituitary and adrenal cortex activity has already demonstrated in *Gerbillus tarabuli* (Zatra *et al*., 2018), *Psammomys obesus* (Benmouloud *et al*., 2014) and *Meriones libycus* (Aknoun-Sail *et al*., 2017). A recent study concluded that testosterone regulates adrenal homeostasis in the Saharan jird *Meriones libycus* by inhibiting β-catenin signaling (Aknoun-Sail *et al*., 2023). In addition, we previously reported modifications induced by testosterone deprivation followed by androgen replacement therapy on the structure and endocrine activity of the adrenal cortex and the adrenocortical immuno-localization of androgen receptors in the free-living male Tarabul's gerbil (Zatra *et al*., 2018). In order to complete these results we targeted in the present paper, the effects of testicular androgens on corticotroph cells and the regulation of adrenocortical homeostasis in this gerbil living in the Algerian Sahara desert. Therefore, we assessed the adrenal cortex remodeling and homeostasis by studying the modifications induced by testicular androgens on the Wnt/b-catenin distribution and the androgen receptors localization in the pituitary-adrenal axis. The effects of testicular androgens upon the activity of this axis were performed by castration experiments applied during the breeding season (spring), followed by testosterone replacement. This study aims to show whether the inhibitory pathway of testosterone involves a central action at the pituitary level and/or a direct action at the adrenal level via the androgen receptor and Wnt/β-catenin signaling.

MATERIAL AND METHOD

Ethical statement. The Animal Care Committee of the Algerian Ministry of Higher Education and Scientific Research approved the experiments in accordance with FELASA guidelines (Executive Decree n° 10-90, which completes the Executive Decree n° 04-82 of the Algerian government, establishing the terms and approval modalities of animal welfare in facilities). Additionally, it was supported by the Algerian Association of Experimental Animal Sciences (Agreement number 45/DGLPAG/DVA.SDA.14) and approved by the USTHB University Local Institutional Animal Care Committee on June 15, 2021, with decision number CEEA-USTHB-19-2024/11119.

Animals and captive conditions. *Gerbillus tarabuli* (Thomas, 1902), commonly named Tarabul's gerbil, belongs to the Gerbillinae subfamily. We used 24 adult males live-trapped using Sherman traps from wild communities in the Béni Abbès region (30° 07' N., 2° 10' W.; 497m of altitude) located in the southwest of Algeria. Trapping was carried out in February-March when animals were sexually active. Just after trapping, females and juvenile males were released back into the wild near their burrows. All animals were housed in individual cages (50 x 35 x 30 cm) with sawdust and sand layers that were regularly renewed. The animal facility was constantly maintained at about 22 ± 2°C temperature and an artificial light of 12 :12 h light-dark cycle. Animals were given daily barley, dates and bread as well as carrots and green vegetables as water sources. Animals were provided seven days for acclimation.

Experimental design. Adult male gerbils are randomly assigned to three experimental groups of 8 individuals: sham-operated group (C/Control), gonadectomized group (GDX) and testosterone-replaced gonadectomized group (GDX+T). Prior to surgery, the animals were anesthetized

with 10 mg/kg administered i.p. of hydrochloride ketamine (Ketalar, Pfizer, NY) in combination with 10 mg/kg i.p. of xylazine (Xylamax, Bimeda-MTC, Canada). The sham-operated group underwent a ventral midline incision without removing the testes. In gonadectomized groups, 16 male gerbils were surgically bilaterally gonadectomized and the testes were quickly removed after ligation of the spermatic arteries. The animals were watched till they gained consciousness and then returned to their cages. In this study, all animals were considered recovered within 24 h of castration. In the GDX+T group, gerbils were treated with testosterone for seven days (from 30 to 37 days after castration); each animal received an intramuscular injection of 75 µg of testosterone enanthate (Androtardyl, Bayer Healthcare, Germany) /40µL sesame oil/100 g body weight twice daily; control and GDX groups received sesame oil as vehicle alone. All the animals are weighed prior to euthanasia (between 09:00 and 11 :00 am), which is performed under deep anesthesia one day after the last injection in the testosterone-treated group. The pituitary gland and both the adrenal glands are quickly removed, stripped of the surrounding fat and weighed before being subjected to adequate processes. The pituitary gland and right adrenal gland are fixed in 0.1 M formalin for 24 h and then subjected to either histomorphometry and immunohistochemistry (b-catenin in adrenocortical tissue) or to immunofluorescence (AR and ACTH in corticotrophs) studies. The seminal vesicles of all groups are taken out and weighed. Weight data are given as relative to 100 g body weight.

Immunofluorescence of androgen receptors AR and ACTH cells in the pituitary

Biochemical methodology. Double-labelled indirect immunofluorescence staining is performed for AR and ACTH cell detection in the pituitary sections. Adjacent sets of 5 µm thick pituitary sections for each group of animals are deparaffinized, rehydrated and washed in 0.1 M Phosphate-Buffered Saline (PBS). The sections are placed in a pressure cooker for antigen unmasking using 0.01 M citric buffer at pH 6. After the step of washing, the pituitary sections were incubated in the presence of horse serum for 20 min at room temperature. Then, they were incubated in a wet chamber with primary antibodies at 4 °C overnight. The two primary antibodies that were used are: 1) a rabbit polyclonal anti-AR antibody (N20; sc-816) previously defined at a dilution of 1: 300 in 0.1 M PBS and 2) a mouse monoclonal antibody against ACTH (sc-69648) at a 1:50 dilution in 0.1 M PBS. The obtained sections were afterwards incubated for 1 h with the secondary antibodies at a 1:500 dilutions (goat anti-rabbit IgG-CFL 555 for AR and goat anti-mouse IgG-CFL 488 for ACTH). Finally, the sections were mounted in an aqueous medium (Vectashield) and the coded sections were examined with a microscope equipped with epifluorescence optics. ACTH cells visualized with the fluorescein filter appear in green; AR cells visualized with the rhodamine filter appear in red. The initial antibodies were omitted to create negative controls that did not show any fluorescence.

Quantification of immunofluorescence. Photographs were used for the morphometric measurement's exploration using Target Cell software to count ACTH cells in an area of 7558.92 μm^2 (the total area of the grid used) and Axio Vision software for ACTH cell area measurements. The cellular immunofluorescence intensity of AR and ACTH was deduced using ImageJ 1.46r software, applied separately to the fluorescent cells for each experimental group as follows:

CTCF = Integrated density - (Selected cell area x Average background fluorescence)

with CTCF = Correlated Total Cell Fluorescence

Background = average fluorescence measured in non-specific immunolabeled regions (the minimum of 3 measures).

Immunohistochemistry of Wnt/β-catenin in the adrenal cortex. The adrenal sections were rehydrated in a water-graded ethanol series after being deparaffinized with xylene in order to detect the presence of β-catenin in the adrenal tissue. The procedure employed on paraffin-embedded tissues that had been unmasked with 10 mM sodium citrate and 0.05 % Tween. (a specific primary monoclonal mouse antibody). We used 3 % H_2O_2 and a serum-free protein block in order to impede the action of endogenous peroxidase. After that, β-catenin antibody (BD610153, a monoclonal mouse antibody, supplied by BD Biosciences) at a dilution of 1:500 was added to the adrenal sections and incubated for an additional night. Signal Stain Boost HRP-Polymer solution (#8114S or #8125P, Cell Signaling) was used to detect primary antibodies. Hematoxylin was used as a counterstain after the signals were produced using the DAB + substrate chromogen technique (Dako). The number of β-catenin-positive adrenocortical cells was counted by Cell Target XE (Universidad de Alcala, Spain) as previously described (Zatra *et al*., 2018).

Statistical analysis. Data values are given as means ± Standard Error of the Mean (SEM). Shapiro-Wilk tests were used to confirm normality and equality of variances following data collection. The graphs were created using GraphPad PRISM Trial version 8.0.2 (263) software. Using SPSS version 23, a one-way analysis of variance (ANOVA) and a post hoc Tukey multiple comparison tests were applied

across treatment groups for each experimental group to determine the statistical significance of the differences. A 5 % threshold was maintained for the significance of the differences.

RESULTS

Effects of gonadectomy and testosterone treatment on the body, seminal vesicles, pituitary and adrenal glands weights. Figure 1 shows the effects on body (1a), seminal vesicles (1b), pituitary (1c) and adrenal (1d) weights induced by testicular removal and testosterone replacement therapy. Castration performed during the breeding season induces 30 days later, a significant body weight decrease (12.5 %; $p<0.05$) and a drastic loss (83.8 %; $p<0.001$) in the relative weight of the seminal vesicles known as the main androgens target, confirming the testosterone falling in the surgically castrated males. For the pituitary and adrenal weights, androgen deprivation induces significant increase of 99.3 % ($p<0.01$) and 63.1 % ($p<0.01$), respectively. Seven days of testosterone replacement restores seminal vesicles and pituitary weights to control values but induces no significant changes in the body mass and adrenal weight ($p>0.05$).

Co-localization of AR and ACTH cells in the anterior pituitary. Double immunolabeling of the anterior pituitary gland of *Gerbillus tarabuli* showed immunoreactive corticotroph nuclei for androgen receptors in all experimental groups. In the control group (Fig. 2a C), only a few ACTH cell nuclei were AR-positive. After castration (Fig. 2a GDX), the ACTH cells were less numerous and only a few labelling of AR was detected in pituitary cells. Quantitatively, we found reduced mean ACTH and AR CTCF values (Fig. 2b) in GDX group compared to control. Testosterone replacement restores the control status (Fig. 2a GDX+T) with some ACTH cells, sometimes with AR-stained nuclei, showing a particular increase in the mean AR CTCF value (Fig. 2b) probably due to AR detection in other pituitary cells than corticotrophs, especially in gonadotrophs.

Immunolocalization of b catenin in the adrenal cortex. In control gerbils, β catenin is immunolocalized mainly at the capsular level (78.9±0.5) % and appeared less important in the three cortical zonae (14.03±2.0 %, 10.25±0.1 % and 12.03±2.2 % in zG, zF and zR respectively) (Table I, Figs. 3 and 4 a, d, g). Gonadectomy induced a strong modification in the cortical repartition of β-catenin. Indeed, it becomes

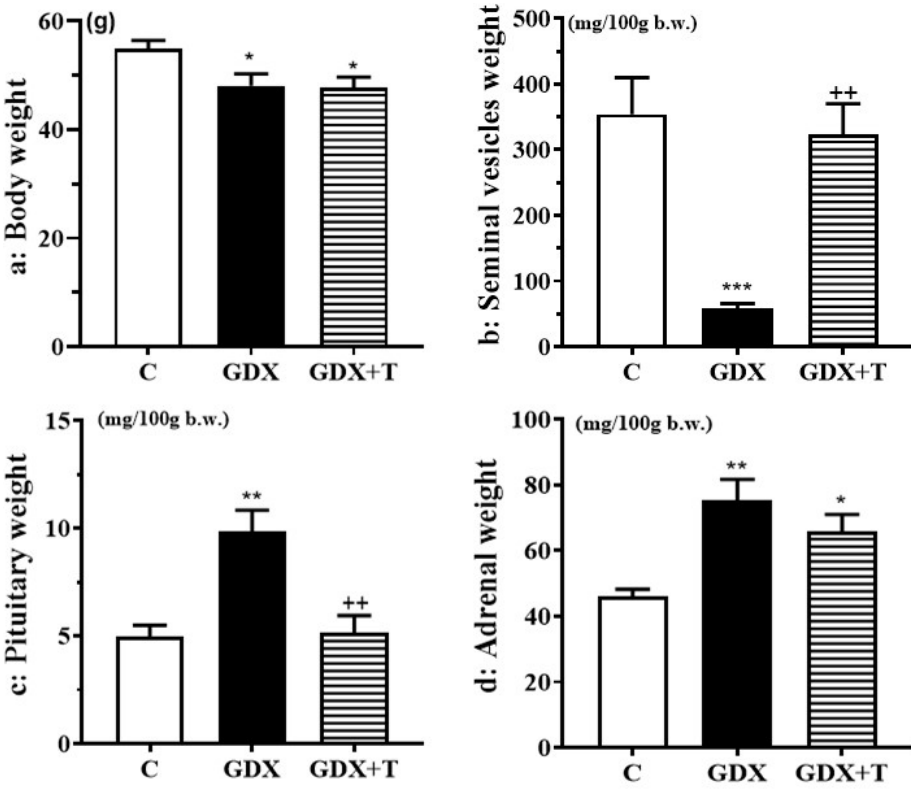

Fig. 1. Effects of castration on body weight (1a) and some organs' weight (1b: seminal vesicles; 1c: pituitary; 1d: adrenal glands) in the adult male *Gerbillus tarabuli.* C, sham-operated; GDX, gonadectomized; GDX+T, gonadectomized-testosterone replaced; bw, body weight. Significance shown as follows: *: vs C; * $p<0.05$; ** $p<0.01$; *** $p<0.001$. +: vs GDX; + $p<0.05$; ++: $p<0.01$; +++: $p<0.001$.

internalized where it forms a net immuno-labelled trail in the zG (52.4±3.5 %), in the zF (45.4±1.7 %) and in the inner zR (36.4±3.7 %) (Table I, Figs. 3 and 4 b, e, h). Testosterone treatment restores the control state with strong immunostaining noticeable only in the capsular (Table I, Figs. 3 and 4 c, f, i).

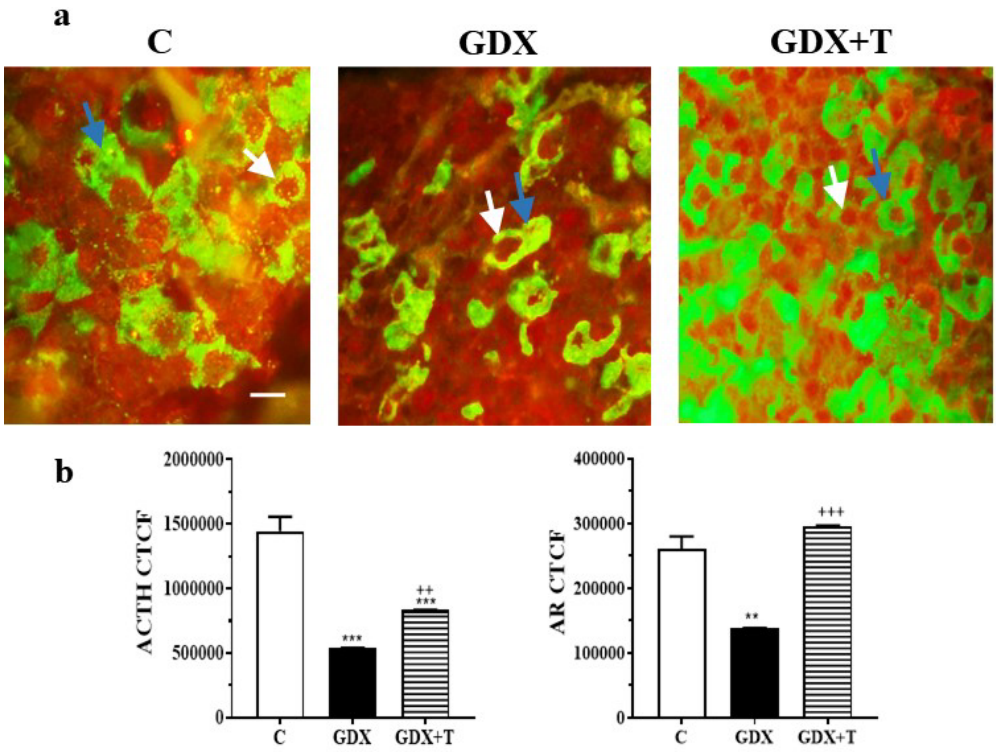

Fig. 2. Immunofluorescence of androgen receptors (AR) and ACTH cells in the anterior pituitary gland in the adult male *Gerbillus tarabuli*. (2a), Co-localization of AR and ACTH cells: Scale bar: 25 µm; white arrowheads for AR (red fluorescence: rhodamine filter) and blue arrowheads for ACTH cells (green fluorescence: fluorescein filter); (2b), Variations of the measured CTCF (corrected total cell fluorescence using ImageJ software) for both AR and ACTH cells. C, sham-operated; GDX, gonadectomized; GDX+T, gonadectomized-testosterone replaced; bw, body weight. Significance shown as follows: *: vs C; * $p<0.05$; ** $p<0.01$; *** $p<0.001$. +: vs GDX; + $p<0.05$; ++: $p<0.01$; +++: $p<0.001$.

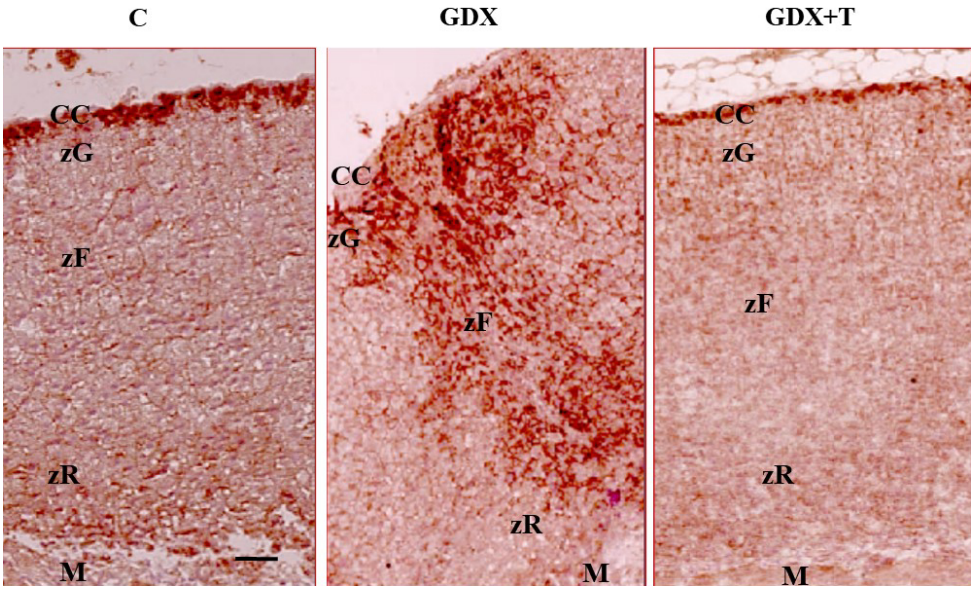

Fig. 3. Immunohistochemistry of β-catenin in the adrenal cortex of the adult male *Gerbillus tarabuli*. Scale bar: 100 µm; Effects of gonadectomy (GDX) and testosterone treatment (GDX+T) compared to sham-operated control (C). CC, conjunctive capsular; zG, zona glomerulosa; zF, zona fasciculata; zR, zona reticularis; M, medulla. Gonadectomy induced a strong modification in the distribution of β-catenin, which becomes internal and forms a weakly immuno-labelled trail in the two outer zonae and appears more intensive in the inner zR.

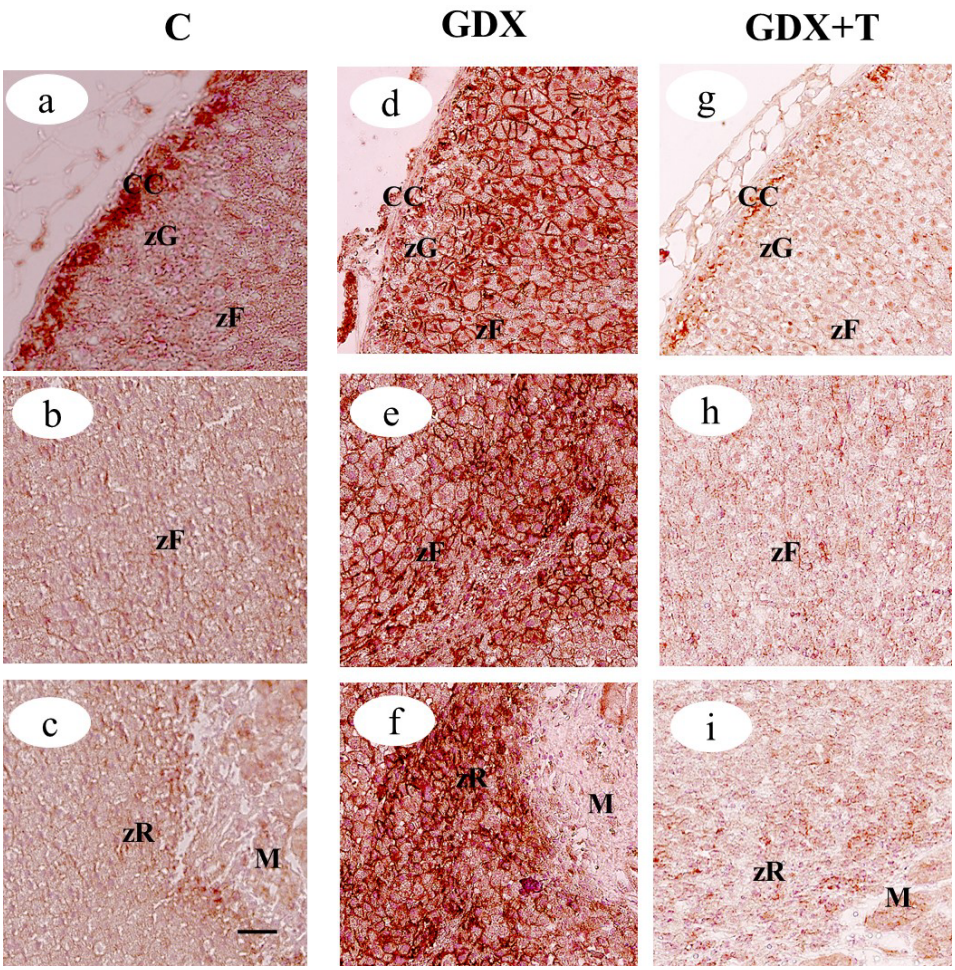

Fig. 4. Immunohistochemistry of β-catenin in the adrenal cortex of the adult male *Gerbillus tarabuli.* Scale bar: 20 µm; Effects of gonadectomy and testosterone treatment compared to sham-operated control. C: sham operated Control (a, b, c); GDX: gonadectomized (d, e, f) ; GDX + T: gonadectomized testosterone replaced (g, h, i). zG: zona gIomerulosa; zF: zona fasciculata; zR: zona reticularis.

Table I. Immunohistochemical quantification of β-catenin reactivity in the adrenal cortex of *Gerbillus tarabuli.* Effect of gonadectomy and testosterone replacement.

Adrenal Cortex Zonae	β-catenin immunoreactivity (%)		
	C	GDX	GDX+T
Capsular	78.9±0.5	11.02±0.8**	54.6±1.4 ++
zG	14.03±2.0	52.4±3.5*	19.5±4.8 +++
zF	10.25±0.1	45.4±1.7***	18.4±1.9 +++
zR	12.03±2.2	36.4±3.7***	15.5±4.10+

Control: sham operated; GDX: gonadectomized; GDX + T: gonadectomized testosterone treated. TC: total adrenal cortex; zG: zona glomerulosa; zF: zona fasciculata; zR: zona reticularis. *: vs Control; +: vs GDX. *, +: $p < 0.05$; **, ++: $p < 0.01$; ***, +++: $p < 0.001$.

DISCUSSION

Gerbillus tarabuli, living in the Sahara Desert, is a seasonal breeder with sexual activity limited to the period of the year that allows birth at the most favorable time for offspring survival. We have previously reported that during this period plasma testosterone levels in intact adult male individuals were higher in early spring (0.87 ng/mL) and fall to lower levels (0.38 ng/mL) in summer and autumn (Zatra *et al*., 2018). Pituitary-adrenal axis activity is negatively correlated with reproductive activity in males of *Gerbillus tarabuli* (Zatra *et al*., 2018) as well as in

Psammomys obesus (Benmouloud *et al*., 2014) and *Meriones libycus* (Aknoun-Sail *et al*., 2017, 2023) living in the same desert area. In these three Saharan species, castration reduced the height of zona fasciculata and cortisol plasma levels while causing an increase in glandular cortisol. This has also been reported in other rodents (Goel *et al*., 2014). This finding suggests that the androgens have an inhibitory effect on the pituitary adrenal axis activity. The study aimed to examine differences in AR expression at the central level (pituitary) and the peripheral level (adrenals) caused by the removal of testicles and subsequent testosterone replacement therapy. Androgen receptors are found in the three adrenocortical zones of the adrenal cortex, according to several studies reported in rats (Trejter *et al*., 2015; Ajdzanovic *et al*., 2016) and gerbils (Benmouloud *et al*., 2014; Aknoun-Sail *et al*., 2017; Zatra *et al*., 2018). However, they are present with low signal in the inner zona reticularis of the intact animal, as reported in the sand rat living in the same region (Benmouloud *et al*., 2014). After castration, AR positivity is more abundant in all the nuclei of the adrenal cells, particularly in those of the zona reticularis, which shows cellular and nuclear hypertrophy that may be related to the increased production of androstenedione, as previously reported in *Gerbillus tarabuli* (Zatra *et al*., 2018). This result indicates a stimulated mitotic activity in this area, similar to the work reported in the sexually inhibited *Peromyscus maniculatus* mouse (Cherry *et al*., 2002), which associates a significant development of juxtamedullary connective tissue infiltrating the reticularis cells. A similar result was also found in the andropause model rat (Ajdzanovic *et al*., 2016).

The study of Pihlajoki *et al*. (2015) showed the existence of progenitors' cells at the capsular, subcapsular and juxtamedullary zones of the adrenal involved in the remodeling of the different layers of the adrenal cortex in response to extreme physiological demands. This can be manifested by cellular zonation, such as the transformation of glomerular cells into fascicular and/or reticular cells under the control of numerous endocrine (ACTH, angiotensin II and LH) and paracrine (fibroblast growth hormone, sonic hedgehog SHH and Wnt/β-catenin) factors. Therefore, gonadal hormone receptors allow sex hormones to influence HPA axis activity as well as quickly and gradually through both conventional and non-classical mechanisms by inhibiting adrenal steroidogenesis enzymes (Stalvey, 2002). In addition, androgens act not only at the peripheral level but also at the central level by modulating regulatory factors such as the hypothalamic CRH or pituitary ACTH production or hypothalamic ADH and oxytocin (Panagiotakopoulos & Neigh, 2014). At this level, unlike the effects on the adrenal gland, castration induced the opposite effects in *Meriones libycus* living in the same arid area. Indeed, while in *Meriones libycus* cortisol and ACTH production increased after castration and returned to normal after testosterone treatment, in *Gerbillus tarabuli*, the same increase in cortisol production observed was associated with a decrease in ACTH production (Aknoun-Sail *et al*., 2017; Zatra *et al*. 2018). These results are supported by the immunohistochemical changes induced on corticotrophs as well as on the AR immunoreactivity in the pituitary, also reported in *Meriones libycus* (Aknoun, 2018) and male mouse (O'Hara *et al*., 2015).

The literature reported contradictory effects depending on the species, such as in the castrated sand rat (Benmouloud *et al*., 2014) or the rat model of andropause (Ajdzanovic *et al*., 2016). Androgens can increase the sensitivity of the hypothalamic-pituitary axis to glucocorticoids by increasing the expression of glucocorticoid receptors (GRs), thereby activating the feedback mechanism exerted on ACTH (Cherry *et al*., 2002). The decrease in ACTH production in castrated *Gerbillus tarabuli* could thus be attributed to the removal of the testosterone-induced inhibition on GR. On the other hand, in *Meriones libycus* (Aknoun, 2018), the inhibitory action of testosterone on ACTH production would be exerted by regulating both hypothalamic CRH and ADH of the parvocellular neurons of the PVNs as reported in rats (Panagiotakopoulos & Neigh, 2014) treated with an androgen antagonist. Indeed, this hypothesis is corroborated by the fact that Desert mammals are known to produce significantly high levels of ADH in response to environmental heat stress. b-catenin's immunohistochemical investigations revealed that it is mainly found in the peripheral cortex (capsule).

According to recent papers, high levels of Wnt/β-catenin signaling within the zG positively regulate the transcription of zG-specific genes, such as aldosterone synthase, and negatively regulate the transcription of zF-specific genes, such as 11β-hydroxylase, in order to determine zonal identity (Kim *et al*., 2008; Drelon *et al*., 2016). The master transcription factor SF1 (NR5A1) is expressed by all adrenocortical cells. The differentiation of SF1+ cells into the zona fasciculata (zF) is prevented by Wnt/β-catenin. This antagonizes the pituitary ACTH, and as a result, the zF expands and differentiates to release glucocorticoids (Mohan *et al*., 2023). Although zF-specific gene expression is inhibited by high Wnt/β-catenin activity, it was proposed that this inhibition occurs along a centripetal gradient when PKA signaling is activated and zG cells simultaneously differentiate into zF cells (Little *et al*., 2021). Regardless of its impact on cellular proliferation, the canonical Wnt/β-catenin pathway's activation prevents cellular differentiation and cell-fate commitment, resulting in tissue hyperplasia (Pignatti *et al*., 2020). Very few studies have examined the effects of gonadectomy on β-catenin

distribution. However, it was demonstrated that gonadectomy makes males more susceptible to hypercorticism and reticular-like formation, whereas testicular androgens boost Wnt signaling that antagonizes PKA, resulting in slower adrenocortical cell turnover and a delayed phenotype (Dumontet *et al*., 2018). Additional information regarding the role of testicular androgens in regulating adrenal activity should be provided by studies employing assays for androstenedione and immunoblotting of β-catenin throughout the adrenal gland as suggested by Akn oun-Sail *et al*.(2023).

One of the non-reproductive organs with the greatest sexual dimorphism is the adrenal gland. For instance, women are more likely to have various types of adrenocortical hyperplasia and neoplasia linked to endocrine manifestations, such as Cushing's disease, than men (Lyraki *et al*., 2023). The molecular processes behind AR's regulation of steroidogenic cell proliferation are less clear despite the genetic evidence supporting this function. It has previously been proposed that Wnt/b-catenin signaling and AR directly oppose one other in epidermal stem cells (Kretzschmar *et al*., 2015). Furthermore, there was no proof discovered that the global b-catenin signaling in male versus female adrenal glands was reduced based on sex (Lyraki *et al*., 2023). Conversely, males' inner cortex showed a consistently higher level of Axin2 expression, a known marker of Wnt/β-catenin signaling, than females', and this increase was caused by DHT administration. This finding is consistent with works which suggested that androgens reverse PKA signaling and cortical cell turnover by favorably influencing Wnt signaling in the adrenal cortex (Dumontet *et al*., 2018).

CONCLUSION

This study enables us to conclude that in the free-living Saharan gerbil *Gerbillus tarabuli*, immunohistochemistry of AR, both at central and adrenal levels and that of β-catenin in the adrenal cortex, shows that testicular androgens exert an inhibitory action on glucocorticoid activity. They act via their specific receptors AR and modulate the Wnt/β-catenin signaling pathway that maintains homeostasis in the adrenal cortex. Therefore, androgens inhibit this pathway in the inner zone of the cortex, in particular the zR, and stimulate the Wnt/β-catenin pathway in the outer zone, promoting the proliferation of progenitor cell capsids. However, androgens did not primarily promote the central nervous pathway. So, subsequent analysis of other molecular markers, such as glucocorticoid, estrogen and LH receptors, steroidogenesis enzymes and apoptotic or cell proliferation markers seems necessary to elucidate the mechanisms that regulate the seasonal endocrine cycles that enable this Saharan gerbil to survive and reproduce in its extremely arid environment.

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RESUMEN: Anteriormente, informamos que el macho en libertad, *Gerbillus tarabuli*, presenta variaciones estacionales opuestas en la actividad del eje hipofisiario-suprarrenal a la del eje hipofisiario-gonadal, lo que sugiere interrelaciones negativas entre estas funciones endocrinas. Por lo tanto, en este artículo investigamos el papel de la testosterona en el eje hipofisiario-suprarrenal mediante la extirpación de testículos durante el período sexualmente activo. Los jerbos fueron capturados en el invierno y a principios de la primavera en su biotopo natural del Sahara. La investigación involucró a tres grupos de ocho machos adultos (control simulado operado, jerbos gonadectomizados y jerbos gonadectomizados con reemplazo de testosterona). Las glándulas suprarrenales y pituitarias derechas se extrajeron rápidamente después de la eutanasia realizada 30 días después de la gonadectomía o 7 días después del reemplazo de testosterona. Luego, se fijaron para la inmunohistoquímica de beta-catenina (β-catenina) en las glándulas suprarrenales utilizando un anticuerpo específico de β-catenina o inmunofluorescencia indirecta de doble marcado para los receptores de andrógenos (AR) y la detección de células ACTH en las secciones de la glándula pituitaria utilizando un anticuerpo policlonal de conejo anti-AR y un anticuerpo monoclonal de ratón contra ACTH. En los jerbos sin intervención, se encontró AR colocalizado con ACTH en las células corticotropicas pituitarias. La inmunolocalización corticosuprarrenal de la β-catenina fue particularmente capsular y ligeramente glomerular, mientras que estuvo ausente en las zonas corticales internas. Tras la orquiectomía, las células ACTH fueron menos numerosas con una expresión débil de AR. En la corteza suprarrenal, la castración indujo una fuerte inmunorreactividad de la β-catenina en las zonas fasciculada y reticular con un gradiente centrípetamente decreciente; la inmunorreactividad de la β-catenina desapareció por completo en la cápsula suprarrenal. La terapia de reemplazo de testosterona restableció todos los parámetros. Estos resultados sugieren un efecto inhibidor de la testosterona sobre el eje hipófisis-suprarrenal de *Gerbillus tarabuli*; este esteroide podría actuar localmente a través de una vía central, con una posición importante en la señalización canónica Wnt/β-catenina, para preservar la zonificación y la homeostasis de la corteza suprarrenal durante todo el año en este roedor sahariano de reproducción estacional.

PALABRAS CLAVE: Jerbo sahariano; Homeostasis suprarrenal; Células ACTH; β-catenina; Dependencia de andrógenos.

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