Stress-induced glucocorticoids alter the Leydig cells’ timing and steroidogenesis-related systems

Meder M., Andric S., Kostic T.
Laboratory for Chronobiology and Aging, Laboratory for Reproductive Endocrinology and Signalling, Centre of Excellence CeRES, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia

Abstract
The study aimed to analyze the time-dependent consequences of stress on gene expression responsible for diurnal endocrine Leydig cell function connecting them to the glucocorticoid-signaling. In the first 24 h after the stress event, a daily variation of blood corticosterone increased, and testosterone decreased; the testosterone/corticosterone ratio was lowest at the end of the stress session overlapping with inhibition of Leydig cells’ steroidogenesis-related genes (Nrx1c1, GR, Hsd11b2, Star, Cyp17a1) and changed circadian activity of the clock genes (the increased Bmal1/Bmal2 and Per2/Per1/Per2 and decreased Cry1 and Rev-erba). The glucocorticoid-treated rats showed a similar response. The principal-component analysis (PCA) displayed an absence of significant differences between treatments especially on Per1 and Rev-erba, the findings confirmed by the in vivo blockade of the testicular glucocorticoid receptor (GR) during stress and ex vivo treatment of the Leydig cells with hydrocortisone and GR-blocker. In summary, stressful stimuli can entrain the clock in the Leydig cells through glucocorticoid-mediated communication.

Experimental design
Adult, three-month-old male Wistar rats were used for the experiments. Three in vivo experimental approaches were applied (1) to examine the effect of immobilization stress (IMO) on Leydig cell activity, IMO was applied from ZT0-3 followed by the expression/functional study in hours after IMO session (ZT3, ZT11, ZT17, and ZT23); (2) to prove that glucocorticoids mimic IMO effects on the Leydig cell function, rats were treated orally with Desoxin (Galenika, SRB; 0.01mg/50 µl/100 g BW) at ZTO and the effects were analyzed after treatment in ZT3, ZT11, ZT17, and ZT23, respectively. Control rats received 50 µl of water simultaneously as experimental rats received Desoxin; (3) the contribution of glucocorticoids in the effects of stress was analyzed by local glucocorticoid receptor blockade. Rats received antagonist of glucocorticoid receptor RU486 (20 µg/20 µl DMSO/testis) in each testis, 12h before the morning IMO session (ZT0-3), and the effects were analyzed immediately after IMO. Control rats received 20 µl DMSO/ testis at the same time as RU486 treated rats; (4) To obtain information about the direct effect of glucocorticoids on peripheral Leydig cells’ clock and steroidogenesis, ex vivo treatment of primary culture with hydrocortisone and/or with RU486 (glucocorticoid receptor antagonist) was performed. The doses of desoxin and hydrocortisone used in the in vivo and ex vivo experiments were selected on the basis of an approximation to the level of glucocorticoids in the blood after stress.

Results
Fig. 4. Treatment with glucocorticoids changed transcription rhythm of steroidogenesis-related genes. Rats were treated per os with Desoxin (10 mg/kg) w/ DMSO (20 µg) and IMO (10 mg/kg) at ZTO and effects were analyzed after treatment in ZT3, ZT11, ZT17, and ZT23. Leydig cells were purified, RNA isolated, and used for RT followed by 96 well plates with clock gene expression (CLOCK, BMAL1, and PER2) and testosterone production. The testicular mRNA levels of genes (CLOCK, Bmal1, Per2, and Cyp11a1) were determined by western blot. Data points for each treatment are represented in an oval. Statistical significance between control and IMO groups for the same time point (p < 0.05). Statistical significance between the groups (including all time points) (p < 0.001). Statistical significance between the groups (including all time points) (p < 0.05).

Fig. 5. Treatment with glucocorticoids changed transcription rhythm of clock genes. Rats were treated per os with Desoxin (10 mg/kg) w/ DMSO (20 µg) and IMO (10 mg/kg) at ZTO and effects were analyzed after treatment in ZT3, ZT11, ZT17, and ZT23. Leydig cells were purified, RNA isolated, and used for RT followed by 96 well plates with clock gene expression (CLOCK, BMAL1, and PER2) and testosterone production. The testicular mRNA levels of genes (CLOCK, Bmal1, Per2, and Cyp11a1) were determined by western blot. Data points for each treatment are represented in an oval. Statistical significance between control and IMO groups for the same time point (p < 0.05). Statistical significance between control and IMO groups for the same time point (p < 0.05). Statistical significance between the groups (including all time points) (p < 0.001). Statistical significance between the groups (including all time points) (p < 0.05).

Conclusion
In summary, stressful stimuli can entrain the clock in the Leydig cells through glucocorticoid-mediated communication.

This work was supported by the MS173057 and APV265. Corresponding author: tatjana.kostic@dbf.uns.ac.rs