VETERINARSKI ARHIV 86 (6), 841-856, 2016

Alterations of hypothalamic RF-amide related peptide-3 and Kiss1 gene expressions during spermatogenesis of rat in chronic stress conditions

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ABSTRACT

The effects were evaluated of chronic stress and the glucocorticoid receptor antagonist (RU486) on mRNA expressions of RF-amide related peptide-3 (RFRP-3) in the dorsomedial hypothalamic nucleus (DMH) and Kiss1 in the arcuate nucleus (ARC) of male rats. Twenty-four male rats were allocated to four equal sized groups: the stress, RU486, stress/RU486, and control groups. In the stress group the rats were restrained 1 hour/day for 12 days. In the RU486 group, the rats were injected with RU486 for 12 days. In the stress/RU486 group, the rats were injected with RU486 1 hour before the stress process for 12 days. Relative expressions of RFRP-3 and Kiss1 mRNAs were determined using real-time PCR. The relative expression of RFRP-3 mRNA in

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ISSN 0372-5480
Printed in Croatia

the stress group was higher than that in the RU486 and control rats. The relative expression of RFRP-3 mRNA did not differ between the stress group and the stress/RU486 rats. Furthermore, the relative expressions of Kiss1 mRNA in the stress, RU486, and stress/RU486 groups were less than that of the control rats. The relative expression of Kiss1 mRNA did not differ between the stress, RU486, and stress/RU486 groups. In conclusion, dysfunction in male rat fertility caused by the chronic stress may be the result of the increase in REFP-3 and the decrease in Kiss1 mRNA expression.

Key words: chronic stress, RFRP-3, kisspeptin, hypothalamus, rats

Introduction

It has been well documented that stress suppresses the hypothalamic-pituitary-gonads (HPG) axis function at various levels (CHAND and LOVEJOY, 2011). Despite many studies on the HPG axis under stress conditions, the molecular connection between stress and reproduction is not well understood. Centrally, stress leads to activation of the hypothalamic-pituitary-adrenal (HPA) axis, which leads to inhibition of the HPG activity through suppression of gonadotropin releasing hormone (GnRH) secretion (TELLAM et al., 2000). It has been suggested that glucocorticoids may mediate this inhibitory effect of stress on GnRH (GORE et al., 2006; TILBROOK et al., 2000).

Acute stress is quite adaptive and can help a mammal survive. Otherwise, chronic stress can be very harmful because mammals have not really adapted to deal with chronic stress. Chronic stress modulates GnRH secretion by many mediators released during the time-course of stressful stimuli, and all the mediators released from the HPG and HPA axes act mainly within the hypothalamus and anterior pituitary gland, to mediate a suppressive effect on gonadotropin secretion (RIVIER and RIVEST, 1991). Glucocorticoids have the main role in stress-induced inhibition of reproduction in rats (McGIVERN and REDEI, 1994).

Nonspecific CRF antagonists reverse suppression of the GnRH pulse generator by stress in rats (CATES et al., 2004). Central administration of the urocortin, CRF homologues, decreases LH pulse frequency in a dose dependent manner (LI et al., 2005). Stress hormones, particularly glucocorticoids and CRF, act on the hypothalamus to inhibit GnRH biosynthesis and secretion (TELLAM et al., 1998).

On the other hand, RFamide (Arg-Phe-NH2) related peptide (RFRP-3, encoded by Rfrp), and kisspeptin (encoded by Kiss1) act as an inhibitor and a stimulator of the reproductive system, respectively (ASADI YOUSEFABAD et al., 2013; NOROOZI et al., 2014; SARVESTANI et al., 2014). RFRP-3, that inhibits gonadotropin release, was recently identified and named the gonadotropin-inhibitory hormone (GnIH) (JAHANARA et al., 2014). The functional role of RFRP-3 in suppression of HPG function in mammals has been documented (JAFARZADEH SHIRAZI et al., 2013). In rodents, immunohistochemical analysis showed that RFRP-3-ir cell bodies were localized in the dorsomedial nucleus

of the hypothalamus (DMH) (JAFARZADEH SHIRAZI et al., 2014). The presence of glucocorticoid and corticotropin releasing factor (CRF) receptors has been shown in a large population of RFamid-related-peptide expressing cells in the hypothalamus (KIRBY et al., 2009). The inhibitory action of RFRP-3 on the reproduction system increases by means of glucocorticoids (KIRBY et al., 2009).

Kisspeptin/GPR54 signaling, which includes kisspeptin and its receptor, Kiss1r, is considered when patients, or rodents lacking functional Kiss1 or Kiss1r genes suffer from hypogonadotropic hypogonadism, presenting with low levels of gonadotropins and sex steroids, impaired sexual organ development, and infertility (SALEHI et al., 2013). Within the rodent brain, kisspeptin/Kiss1 mRNA neurons are found in two primary populations: the arcuate nucleus (ARC), and the anteroventral periventricular nucleus and the medial preoptic area (AVPV/mPOA) (ADAVI et al., 2011; YEO, 2013). Kisspeptin increases the electrical firing of GnRH neurons in the hypothalamus (DUMALSKA et al., 2008). In female rats, reduced Kiss1-Kiss1r expression was shown to be a main factor in stress-related decreases of LH secretion (KINSEY-JONES et al., 2009).

Therefore, the present study was designed to evaluate if stress hormones could modulate the expression of RFRP-3 and Kiss1 mRNA via the glucocorticoid receptor. Moreover, the other purpose of the present study was to determine whether 12 days restraint stress, applied to adult male rats, affects the sperm parameters.

Materials and methods

Animals. Twenty-nine adult male Sprague-Dawley rats, with a mean and standard deviation (SD) weight of 236.8 ± 23.5 g, were housed alone in cages under a controlled temperature, 23 ± 1 °C (mean ± SD), a 12 hour light/dark cycle and 55 ± 5% (mean ± SD) relative humidity, at the Center of Comparative and Experimental Medicine, Shiraz University of Medical Sciences, Iran. They had free access to standard pellets and water ad libitum during the experimental period. The experiment was approved by Shiraz University of Medical Sciences ethics committee.

Chronic stress induction. Twenty-four intact adult male rats were randomly divided into four equal groups (n = 6) that is: stress, RU486, stress/RU486, and control groups. In the stress group, the rats were individually restrained for 1 hour sessions in plastic cylinders (20.5 × 8 × 6 cm) with holes for ventilation and their extended tails. The cylinders were just large enough to allow rats of the size used to turn around easily. In the RU486 group, the rats were injected subcutaneously with RU486 (2.5 mg/kg, 20 µL/rat; ab120356, Abcam Ltd, Cambridge, UK) every day for 12 days. In the stress/RU486 group, the rats were injected subcutaneously with the same dose of RU486 one hour before the stress process for 12 days. The control group was allowed to move freely in a laboratory rat cage type III, and did not undergo chronic stress and/or RU486. At the

end of the treatment period, the rats were weighed and then anaesthetized by inhalation of ether.

Testosterone and cortisol evaluations. Trunk blood was collected and serum samples were stored at -70 °C until assayed. Serum testosterone concentrations were evaluated by a testosterone kit (ELISA, Diagnostics Biochem Canada Inc., Ontario, Canada) and the kit sensitivity was 0.022 ng/mL. Serum cortisol concentrations were evaluated by a cortisol kit (RIA, Institute of Isotopes Ltd, Budapest, Hungary), and the kit sensitivity was 2.9 nmol/L.

Evaluation of epididymis sperm quality. The right caudal epididymis of all groups was immediately removed and used for sperm analysis. Briefly, approximately the tail of the epididymis was separated and placed in an Eppendorf tube containing 1.5 mL pre-warmed phosphate buffered saline (PBS), transferred to an incubator and kept at 37 °C for 40 min to facilitate the spontaneous release of sperm from the epididymis. Semen sample slides were evaluated by light microscopy in 10 randomly selected fields with ×40 magnification, to estimate the percentage of motile sperm. The mean sperm counts were calculated. Briefly, the semen samples were diluted with distilled water, and transferred to the Neubauer chamber. Then, the number of sperm was counted in large squares within the central counting area of the chamber. Furthermore, sperm viability was evaluated by use of eosin-nigrosin staining. Briefly, a fraction of each semen sample was mixed with an equal volume of 0.5% eosin-nigrosin solution, and smears on a glass microscope slide were evaluated with light microscopy in 10 randomly selected fields by microscope for the percentage of vital (unstained) and dead (stained) spermatozoa.

RFRP-3 and Kiss1 real-time PCR. Five male rats were used as the castrated control group for real-time PCR. The rats were anaesthetized by an intraperitoneal injection of ketamine (100 mg/kg; Woerden, Netherlands) and xylazine (7 mg/kg; Alfazyne, Woerden, Netherlands) and castrated by means of a ventral midline incision. Further procedures were carried out after a 2-week recovery period.

The brains of the five groups of rats were immediately removed and the diencephalon was dissected out by an anterior coronal section, anterior to the optic chiasm, and a posterior coronal cut at the posterior border of the mammillary bodies. To separate ARC from AVPV, a third coronal cut was made through the middle of the optic tract, just rostral to the infundibulum (SALEHI et al., 2012-2013). The specimens consisting of ARC and DMH were stored in liquid nitrogen until further analysis.

Total RNA was extracted using the Tripure isolation reagent (Roche Life Science, Branford, CT). Briefly, the tissue (100 mg) was ground in liquid nitrogen, transferred to Tripure isolation reagent RNX-Plus buffer (1 mL) in an RNase-free microtube, mixed thoroughly, and kept at room temperature for 5 min. Chloroform (0.2 mL) was added to the slurry, mixed gently and incubated at room temperature for 15 min. The mixture was

centrifuged at 12,000×g (4 °C) for 20 min, and the supernatant was transferred to another tube and precipitated with an equal volume of isopropanol for 15 min. The RNA pellet was washed with 75% ethanol and quickly dried and re-suspended in 50 µL RNase-free water. The integrity and quantity of RNA was checked by visual observation of 28S and 18S rRNA bands on a 1% agarose gel. The purified total RNA was quantified by Nano-Drop ND 1000 spectrophotometer (Nano-Drop Technologies, Wilmington, DE, USA). The DNase treatment was carried out using a DNase kit (Fermentas, St. Leon-Roth, Germany) according to the manufacturer's instructions. The DNase-treated RNA was used for the first strand cDNA synthesis according to the manufacturer's guide (Fermentas, St. Leon-Roth, Germany) in a final volume of 20 µL.

Table 1. Sequences of real time PCR primers and amplification reactions conditions for evaluation of the relative expression of RFRP-3, Kiss1, beta-actin, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes in the rat hypothalamus

Primer	Sense and anti-sense sequence	Amplicon length (bp)	Amplification condition
RFRP-3	AAGACACTGGCTGGTTTG TTGAAGGACTGGCTGGAG	192	15 min at 94 °C, 40 cycles of 94 °C 10 s, 58 °C 15 s, and 72 °C 30 s
Kiss1	GCTGCTTCTCCTCTGTGT TAACGAGTTCCTGGGGTC	107	15 min at 94 °C, 40 cycles of 94 °C 10 s, 58 °C 15 s, and 72 °C 30 s
Beta-actin	CCACACTTTCTACAATGAGC ATACAGGGACAACACAGC	169	15 min at 94 °C, 40 cycles of 94 °C 15 s, 57.8 °C 20 s, and 72 °C 30 s
GAPDH	CAAGATGGTGAAGGTCGGTGTG CGTGGGTAGAGTCATACTGGAA	158	15 min at 94 °C, 40 cycles of 94 °C 10 s, 60 °C 15 s, and 72 °C 30 s

Primers were designed (Table 1), using Allele ID 7 software (Premier Biosoft International, Palo Alto, USA) for reference genes, RFRP-3 (NM_023952) and Kiss1 (NM_181692). The rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene (NM_017008) and beta-actin (NM_031144) were used as reference genes for data normalization. Relative real-time PCR was performed using a real time master mix (Yekta Tajhiz Azma, Tehran, Iran) in a 20 µL volume, containing 1 µL cDNA, 1X SYBR Green buffer and 4 pmol of primer. The amplification reactions were carried out in a StepOne cycler (Applied Biosystems, Foster City, CA, USA) (Table 1). After 40 cycles, the specificity of the amplifications was tested by heating from 60 °C to 95 °C, resulting in melting curves. To ensure that the PCR products were generated from cDNA, and not the genomic DNA, proper control reactions were implemented (using RNA as a sample). For quantitative real-time PCR data, the relative expression of the RFRP-3 or Kiss1

mRNAs was calculated on the basis of the threshold cycle (CT) method. The CT for each sample was calculated using StepOne real-time PCR software (Applied Biosystems, Foster City, CA, USA). Accordingly, the fold expression of the target mRNAs over the reference values was calculated by the equation $2^{-\Delta\Delta CT}$ (LARIONOV et al., 2005), where ΔCT was determined by subtracting the corresponding internal control CT value from the specific CT of the target (RFRP-3 or Kiss1). The ΔΔCT was obtained by subtracting the ΔCT of each experimental sample from that of the calibrator (castrated male control rats).

Statistical analysis. The data of the weight, serum testosterone and cortisol concentrations, the sperm evaluation indices, and the relative expression of RFRP-3 and the Kiss1 gene were subjected to a test of normality and analyzed by one-way ANOVA (SPSS for Windows, version 20, SPSS Inc, Chicago, Illinois), and mean separation was performed by the post hoc LSD test at $P<0.05$. The Spearman correlation test was performed to evaluate the correlation coefficient between the sperm evaluation indices and gene expression.

Results

Weight and testosterone and cortisol concentrations. There was no significant difference in the weights of the rats in all groups (Fig. 1, $P>0.05$). Restraint stress (1 hour/day for 12 consecutive days) significantly decreased serum testosterone levels, and subcutaneous (sc) administration of RU486 (2.5 mg/kg/20μL/rat) blunted the inhibitory effects of chronic stress on serum testosterone concentration ($P<0.05$, Fig. 2A). On the other hand, the chronic restraint stress significantly increased serum cortisol levels, and RU486 administration blunted the stimulatory effects of chronic stress on serum cortisol concentration ($P<0.05$, Fig. 2B).

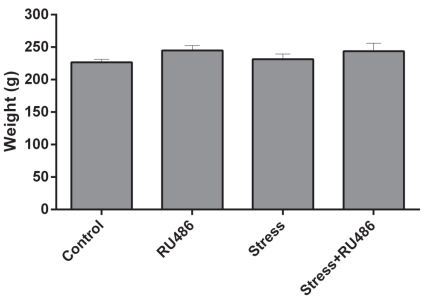

Fig. 1. The mean and standard error of body weight in different groups to evaluate the effects of chronic stress on adult male rats. RU486, glucocorticoid receptor antagonist.

Sperm analysis findings. Although there was an increase in the mean sperm concentration ($\times 10^6$/mL) in the stress group compared with the control group, it was not significant (P>0.05; Fig. 3A). However, there was a significant increase in the sperm concentration ($\times 10^6$/mL) in the RU486 group compared with the stress/RU486 and control groups (P<0.05; Fig. 3A). Moreover, the percentage of motile sperm in RU486, stress, and stress/RU486 groups was lower than in the control group (P<0.05; Fig. 3B). In addition, a reduction was observed in the percentage of sperm viability in the stress group compared with the RU486 and control groups (P<0.05; Fig. 3C).

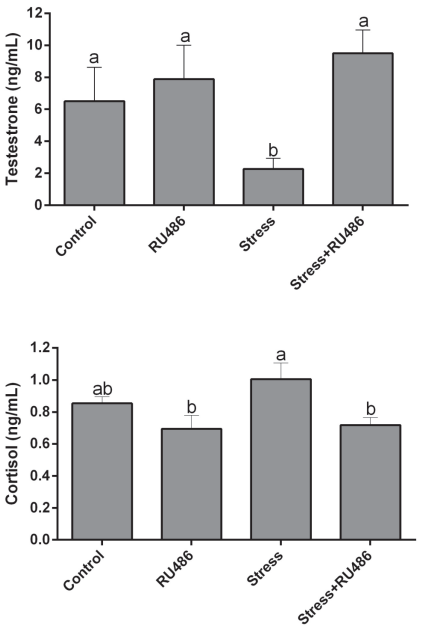

Fig. 2. The mean and standard error of serum A, testosterone and B, cortisol concentrations after chronic restraint stress-induced rats (n = 6). [a,b] Different superscript letters indicate significant differences between groups. RU486, glucocorticoid receptor antagonist.

RFRP-3 and Kiss1 expressions. Relative expression of RFRP-3 mRNA in DMH in the chronic stress group was higher than that of the RU486 and control rats (P<0.05, Fig. 4A). The relative expression of RFRP-3 mRNA did not differ between the stress group and stress/RU486 rats (P>0.05). Furthermore, relative expression of Kiss1 mRNA in ARC in the stress, RU486, and stress/RU486 groups was lower than that of the control rats

(P<0.05, Fig. 4B). Relative expression of Kiss1 mRNA did not differ between the stress, RU486, and stress/RU486 groups (P>0.05). There was a negative correlation coefficient between expression of RFRP-3 mRNA and sperm motility (r = -0.484, P = 0.04).

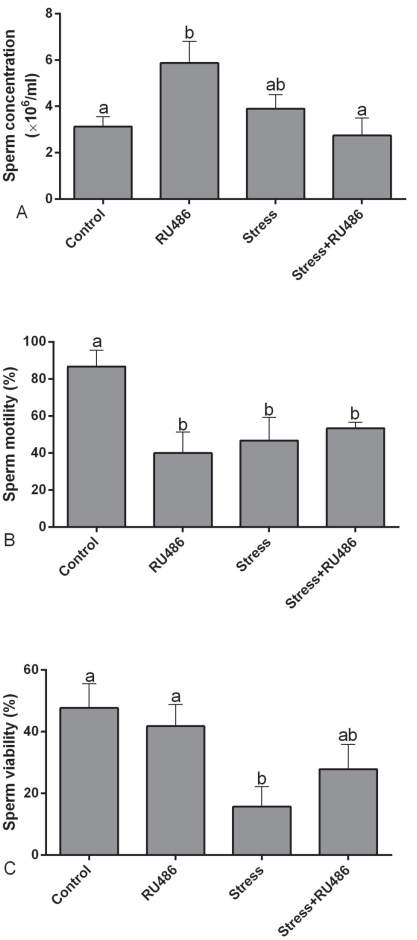

Fig. 3. The mean and standard error of sperm quality evaluation indices, A, sperm concentration (×10⁶/mL), B, percentage of sperm motility, and C, percentage of sperm viability in male rats during chronic stress. [a,b] Different superscript letters show significant differences between groups (P<0.05). RU486, glucocorticoid receptor antagonist.

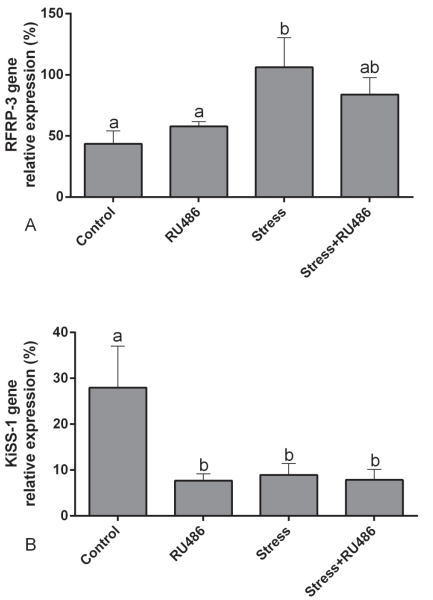

Fig. 4. The effect of chronic stress on the relative expression (mean ± SE) of RFRP-3 mRNA (A) and Kiss1 mRNA (B) in the hypothalamus of male rats (n = 6). [a,b] Different superscript letters show significant differences between groups ($P<0.05$). RU486, glucocorticoid receptor antagonist.

Discussion

The present study showed that chronic restraint stress increased RFRP-3 mRNA levels in the DMH of rats. Consistent with our findings, it has been shown that RFRP-3 expressing cells in DMH, directly regulated by glucocorticoids and stress-mediated transcriptional regulation of RFRP-3 mRNA, exert their effect on the HPG axis (GOJSKA and BELSHAM, 2014; KOTITSCHKE et al., 2009). Moreover, it was shown that GnIH positive neurons increased in sparrows under capture-handling stress conditions (CALISI et al., 2008). Stress-induced increases in adrenal glucocorticoids caused an increase in RFRP that contributed to hypothalamic suppression of reproductive function in male rats (KIRBY et al., 2009). In addition, RFRP-3 neurons co-expressed with glucocorticoid and androgen receptors (POLING et al., 2012). In the present study, RU486 was used as an antiglucocorticoid receptor agent, with the aim of delineating the glucocorticoid mediated effects on the RFRP-3/kisspeptin system, in the presence of chronic stress. It has been reported that in addition to anti-progestational and anti-glucocorticoid activity, RU486 possesses anti-androgen-like effects (HACKENBERG et al., 1996). RU486 induces

an inappropriate conformational change of the receptor, which inhibits interaction with the transcription initiation complex (KUIL et al., 1995). In the current study, blockade of glucocorticoid action by administration of RU486 decreased RFRP-3 expression more than in the stress group, although in the stress/RU486 group, RU486 decreased the effect of stress on RFRP-3 expression to some extent. This finding reveals a glucocorticoid induced increase in RFRP-3.

Moreover, chronic restraint stress decreased Kiss1 mRNA levels in the ARC of rats, which confirms other studies (KINSEY-JONES et al., 2009). It has been shown that kisspeptin neurons express androgen receptors and estrogen receptors, and directly innervate GnRH neurons (OAKLEY et al., 2009). Consistent with our findings, glucocorticoid receptor knock-out mice specifically in kisspeptin-containing neurons, despite a rise in corticosterone, expression of Kiss1 mRNA was not inhibited during restraint stress (WANG et al., 2012). Interestingly, RU486 reduced Kiss1 mRNA expression and in the RU486/stress group could not increase expression of Kiss1 mRNA, and did not reverse the stress inducing effects. Kisspeptin displays a potent stimulatory effect on gonadotropin release, with the administration of kisspeptin resulting in abrupt and sustained LH secretion (OAKLEY et al., 2009). SÁNCHEZ-CRIADO et al. (1999) reported that RU486 inhibited LH secretion at the pituitary level. It is quite possible that our results could be due to the effects of RU486 at the hypothalamic level. Nevertheless, the anti-androgenic effects of RU486 directly on kisspeptin neurons in the ARC nucleus should be elucidated. In addition to glucocorticoid receptors, there is evidence that androgen receptors are also localized in kisspeptin neurons (OAKLEY et al., 2009). Thus, treatment with RU486 was found to decrease kiss1 mRNA expression during chronic restraint stress.

Exposure to chronic restraint stress also decreases sperm motility and sperm viability. Restraint stress acts via glucocorticoid receptors on Leydig cells to suppress the response to gonadotropins in the testes (ORR and MANN, 1992). On the other hand, this study showed the negative effect of stress on kisspeptin expression in the ARC. The fact that kisspeptin is a potent stimulator of LH secretion suggests that reduced sperm quality might be linked to reduced kisspeptin expression. However it seems that direct effects of glucocorticoids at Leydig cells in immobilization stress (DONG et al., 2004). Local intratesticular administration of RU486 partially reversed the immobilization stress-induced decrease in testosterone levels, confirming that glucocorticoid and its receptor are involved in steroidogenic suppression (DONG et al., 2004). On the other hand, in this study, RFRP-3 increase during restraint stress suppressed sperm motility and sperm viability. As described by other researchers RFRP-3 can suppress spermatogenesis by acting at all level of HPG axis of birds and mammals (UBUKA et al., 2014). These data suggest that a possible mechanism underlying adverse effects of chronic stress on sperm quality involves actions of glucocorticoids on the Kisspeptin/RFRP3 system.

Restraint stress decreased serum testosterone levels and increased serum cortisol levels. Consistent with our findings, repeated immobilization stress for 10 days negatively affected testicular steroidogenesis (STOJKOV et al., 2012). Increased glucocorticoids (stress-induced or exogenous administration) exert effects on reproductive system, as glucocorticoid receptors are expressed in various cell types of testis (NIRUPAMA et al., 2013). Furthermore, glucocorticoids directly inhibited enzymes are involved in testosterone biosynthesis (XIAO et al., 2010). Consistent with our results, other studies in animal investigations showed a significant decrease in serum testosterone level following chronic stress (STOJKOV et al., 2012). Increased in apoptosis of spermatogonia and Leydig cells after chronic stress is also reported (KARAMI KHEIRABAD et al., 2016). Corticosterone suppressed the 17β-hydroxysteroid dehydrogenase type 3 (HSD17B3) and 3β-hydroxysteroid dehydrogenase type 1 (HSD3B1) enzymes mRNA expression in Leydig cells and through that decreased steroidogenic activity of testis (BADRINARAYANAN et al., 2006). Production of testosterone in the Leydig cells is vital for the spermatogenesis in the seminiferous tubules (O'HARA and SMITH, 2015), therefore decrease in spermatogenesis indices in the current study was logic.

RU486 pretreatment attenuated the stress-induced alterations in male fertility. Such anti-stress effects of RU486 on the seminiferous tubules appear to closely parallel increases in the serum level of testosterone. The anti-glucocorticoid effect of RU486 is related to masking DNA domain of the glucocorticoid receptor (GROENEWEG et al., 2013), however it appears RU486 to impair nuclear translocation of glucocorticoid receptors maybe due to inefficient release of heat-shock proteins (CZAR et al., 1995). Glucocorticoid receptor blockade by intra testicular administration of RU486, prevented the immobilization-induced decline in plasma testosterone levels (DONG et al., 2004). Therefore, the changes in testosterone suggest a suppression of androgen biosynthesis directly by glucocorticoid or indirectly through HPG axis.

In the present study, these changes in hormonal concentrations and neuropeptides expressions after chronic stress were not associated with change in body weight. In contrast, during acute stress in rats increased in body weight was observed (RYAN et al., 2014). This may reflect changes in element of feeding behavior are not related to chronic stress signals.

Therefore, psychophysical stress causes male subfertility by acting on the testis and decreased enzyme content (STOJKOV et al., 2012) and testosterone production (TURNER and LYSIAK, 2008) or whether due to effect on the higher level in hypothalamic-pituitary axis (KIRBY et al., 2009), it is something all publications deal with to some extent.

Conclusions

Chronic stress was found to suppress expression of two main hypothalamic neuropeptides mRNA, Kiss1 and RFRP-3, related to reproductive system. Systemic administration of RU486 partially reversed the restraint-induce decrease in RFRP-3 mRNA expression, confirming that glucocorticoids and its receptor are involved in steroidogenic suppression.

Acknowledgements

This study was supported by Infertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran. The authors would like to thank the staff of Transgenic Technology Research Center and Also Laboratory Animal Center, Shiraz University of Medical Sciences, for their assistance in this study.

Conflict of interest

There is no conflict of interest.

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Received: 11 June 2015
Accepted: 20 August 2016

KARAMI KHEIRABAD, M., S. AHMADLOO, B. NAMAVAR JAHROMI, F. RAHMANIFAR, A. TAMADON, A. RAMEZANI, M. OWJFARD, F. SABET SARVESTANI, O. KOOHI-HOSSEINABADI: Promjene u RF-amidu srodnom peptidu-3 hipotalamusa i ekspresijama gena Kiss1 tijekom spermatogeneze kod štakora u uvjetima kroničnog stresa. Vet. arhiv 86, 841-856, 2016.

SAŽETAK

Istražen je učinak kroničnog stresa i antagonista glukokortikoidnog receptora (RU486) na ekspresiju mRNA RF-amidu srodnog peptida-3 (RFRP-3) u dorzomedijalnoj jezgri hipotalamusa (DMH), te na ekspresije gena Kiss1 u arkuatnom nukleusu (ARC) štakora. Dvadeset i četiri štakora bila su raspodijeljena u četiri jednake skupine: stresna skupina, RU486 skupina, stresna/RU486 skupina i kontrolna skupina. U stresnoj skupini štakori su 12 dana bili obuzdani tijekom jednog sata dnevno. U skupini RU486, štakorima je tijekom 12 dana bio primijenjivan RU486. U skupini stres/RU486, štakorima je tijekom 12 dana apliciran RU486 jedan sat prije postupka obuzdavanja. Relativne ekspresije RFRP-3 i Kiss1 mRNA određene su lančanom reakcijom polimerazom u stvarnom vremenu. Relativna ekspresija RFRP-3 mRNA u stresnoj skupini bila je veća nego u skupini RU486 i kontrolnoj skupini. Relativna ekspresija RFRP-3 mRNA nije bila različita između stresne skupine i stres/RU486 skupine. Nadalje, relativne ekspresije Kiss1 mRNA u stresnoj skupini, skupini RU486, i stresnoj skupini/RU486 bile su manje u odnosu na kontrolnu skupinu. Relativna ekspresija Kiss1 mRNA nije se razlikovala između stresne skupine, skupineRU486 i stresne skupine/RU486. Zaključno, disfunkcija plodnosti kod štakora izloženih kroničnom stresu može biti uzrokovana putem povećane ekspresije RFRP-3 i smanjene ekspresije Kiss1 mRNA.

Ključne riječi: kronični stres, RFRP-3, kisspeptin, hipotalamus, štakori