Neuroendocrinology Letters Volume 36 No. 3 2015
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

SHORT COMMUNICATION

Effects of LPS injection on the hypothalamic and testicular mRNA expression levels of reproductive factors in male rats

Takeshi Iwasa [1], Toshiya Matsuzaki [1], Altankhuu Tungalagsuvd [1], Munkhsaikhan Munkhzaya [1], Akira Kuwahara [1], Toshiyuki Yasui [2], Minoru Irahara [1]

1 Department of Obstetrics and Gynecology, The University of Tokushima Graduate School, Institute of Health Biosciences, Kuramoto-cho, Tokushima, Japan
2 Department of Reproductive Technology, Institute of Health Biosciences, The University of Tokushima Graduate School, Japan

Correspondence to: Takeshi Iwasa
Department of Obstetrics and Gynecology
The University of Tokushima Graduate School, Institute of Health Biosciences
3-18-15 Kuramoto-cho, Tokushima 770-8503, Japan.
TEL: +81-88-633-7177; E-MAIL: iwasa.takeshi@tokushima-u.ac.jp

Submitted: 2015-06-25 Accepted: 2015-07-25 Published online: 2015-08-15

Key words: **LPS; RFRP; Kiss1; hypothalamus; testis**

Neuroendocrinol Lett 2015; **36**(3):193–195 **PMID:** 26313382 NEL360315A01

Abstract

OBJECTIVES: In the hypothalamus, kisspeptin and RFamide-related peptide (RFRP) regulate gonadotropin-releasing hormone expression. Kisspeptin and RFRP are also found in the testes and might play roles in steroidogenesis and spermatogenesis.

DESIGN AND RESULTS: The present study demonstrated that the hypothalamic mRNA expression level of the kisspeptin receptor was decreased by the injection of lipopolysaccharide (LPS) (500 μg/kg) in male rats, and it was suggested that such changes might contribute to reductions in serum luteinizing hormone levels. Contrary to our expectations, hypothalamic RFRP and testicular GPR147 (the RFRP receptor) mRNA expression were also decreased by LPS injection.

CONCLUSIONS: We speculate that changes in hypothalamic RFRP expression might represent a protective response aimed at attenuating LPS-induced anorectic responses.

INTRODUCTION

It has been reported that stress attenuates hypothalamic-pituitary-gonadal (HPG) axis activity and that such changes lead to the down-regulation of reproductive functions (Iwasa *et al.* 2009). In males, inflammatory stress reduces serum gonadotropin and testosterone levels by attenuating hypothalamic gonadotropin-releasing hormone (GnRH) expression and directly inhibiting testicular steroidogenesis. Recently, it has been revealed that kisspeptin, which is a positive regulator of GnRH expression, and RFamide-related peptide (RFRP), which is a negative regulator of GnRH expression, play important roles in reproductive functions under non-stressed conditions (Khan *et al.* 2012). In addition, it has been reported that kisspeptin (and its receptor) and RFRP (and its receptor) are also expressed in the gonads and might play a role in steroidogenesis and/or spermatogenesis (Zhao *et al.* 2010; Hsu *et al.* 2014).

Several studies have indicated that the hypothalamic activity and/or expression levels of kisspeptin and RFRP are affected by stress; however, these

results are disputed. In a previous study, we showed that the injection of a high (septic) dose of lipopolysaccharide (LPS) resulted in decreased hypothalamic kisspeptin mRNA expression and increased hypothalamic RFRP and GPR147 (the receptor for RFRP) mRNA levels in female rats (Iwasa *et al.* 2014). On the other hand, the injection of a moderate dose of LPS did not affect the mRNA levels of these factors (Iwasa *et al.* 2014), suggesting that kisspeptin and RFRP expression might only be affected by severe inflammatory stress in females. As far as we know, the effects of stress on hypothalamic kisspeptin and RFRP expression in males have not been fully elucidated. In addition, the effects of stress on gonadal kisspeptin and RFRP expression have never been examined. If the expression of these molecules is influenced by stress, such changes might play a role in the effects of stress on testicular steroidogenesis. Thus, in this study we investigated the effects of inflammatory stress on the hypothalamic and testicular expression of kisspeptin and RFRP in male rats.

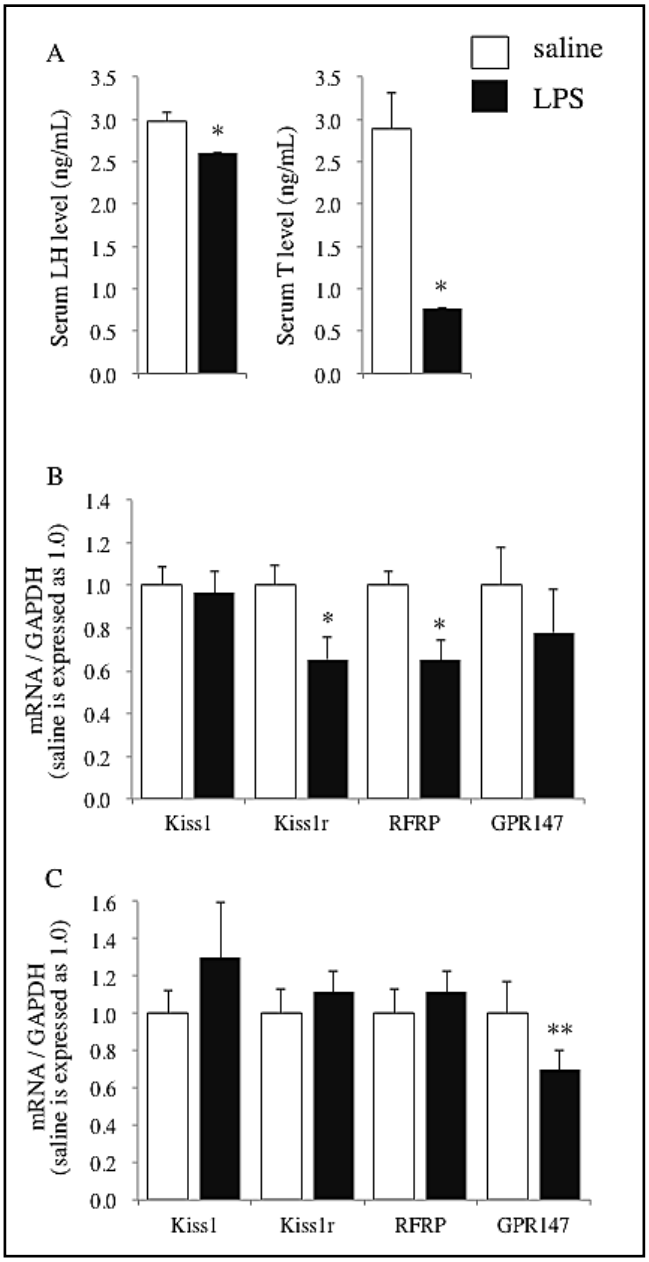

Fig. 1. The serum LH and testosterone levels and hypothalamic and testicular kisspeptin, Kiss1r, RFRP, and GPR147 mRNA expression levels of the saline-injected and LPS-injected groups (n=8 per group). The mRNA levels of the saline-injected group were defined as 1.0. Data are expressed as mean ± SEM values. **p*<0.05, ***p*<0.01.

MATERIALS AND METHODS

Sixteen male Sprague-Dawley rats (Charles River Japan, Tokyo, Japan) were housed under controlled light (12 h light: 12 h darkness) and temperature (24 °C) conditions. All animal experiments were conducted in accordance with the ethical standards of the institutional Animal Care and Use Committee of the University of Tokushima. At 10 weeks of age, eight rats were injected with LPS (026: B6; Sigma, St. Louis, MO, USA; 500 μg/kg, i.p.), and another eight rats were injected with saline. The rats' blood, brains, and testes were collected at 6 h post-injection. The rats' serum luteinizing hormone (LH) levels were measured using radioimmunoassay kits (rat LH [I-125] RIA kit, Institute of Isotopes Co., Ltd., Tokyo, Japan), and their serum testosterone levels were examined using an electrochemiluminescence immunoassay (ECLIA; Roche Diagnostics GmbH, Mannheim, Germany). Hypothalamic explants were dissected from the rats' whole brains as described previously (Iwasa *et al.* 2014). Total RNA was isolated from the hypothalamic and testicular samples and used to synthesize cDNA. Then, the mRNA expression levels of kisspeptin, the kisspeptin receptor (Kiss1r), RFRP, and GPR147 were measured using real-time PCR analysis, as described previously (Iwasa *et al.* 2014). The expression level of each gene was normalized to that of β-actin. The primer sequences and PCR conditions used to assess the expression levels of kisspeptin, Kiss1r, RFRP, and GPR147 were described in our previous study (Iwasa *et al.* 2014). All data are presented as mean ± SEM values. Statistical analyses were performed with the Student's t test or Mann-Whitney U test, as appropriate.

RESULTS AND DISCUSSION

The serum LH and testosterone levels of the LPS-injected rats were lower than those of the saline-injected rats (Figure 1A), indicating that inflammatory stress reduced the activity of the HPG axis. The hypothalamic Kiss1r mRNA levels of the LPS-injected rats were lower than those of the saline-injected rats (Figure 1B). In addition, the hypothalamic RFRP mRNA levels of the LPS-injected rats were lower than those of the saline-injected rats. On the other hand, the hypothalamic kisspeptin and GPR147 mRNA levels of the two groups did not differ. These findings suggest that kisspeptin activity

is reduced via the down-regulation of Kiss1r expression in adult male rats. Contrary to our expectations, the hypothalamic mRNA expression level of RFRP, which suppresses GnRH expression, was also decreased by LPS injection. It is possible that such changes in hypothalamic RFRP mRNA expression represent a protective response against the excessive suppression of the HPG axis. Alternatively, changes in RFRP expression might be involved in LPS-induced anorectic responses because hypothalamic RFRP plays a role in increasing appetite and feeding behavior (Murakami *et al.* 2008). However, further studies are needed to examine these hypotheses.

Previously, we have reported that the injection of a high dose of LPS (5 mg/kg) attenuated hypothalamic kisspeptin mRNA expression and increased hypothalamic RFRP mRNA expression in female rats (Iwasa *et al.* 2014). On the other hand, in the same study the injection of a moderate dose of LPS (500 μg/kg) did not affect the hypothalamic kisspeptin, Kiss1r, RFRP, or GPR147 mRNA expression levels of the female rats. As the same dose of LPS (500 μg/kg) induced hypothalamic Kiss1r and RFRP mRNA expression in the present study, we speculate that hypothalamic kisspeptin and RFRP expression might be more sensitive to LPS-induced inflammatory stress in male rats. Castellano *et al.'s* finding that the repeated injection of LPS (250 μg/kg) attenuated hypothalamic kisspeptin expression supports our suggestion (Castellano *et al.* 2010).

In the present study, the testicular mRNA level of GPR147, but not those of kisspeptin, Kiss1r, or RFRP, were reduced by the injection of LPS (Figure 1C). It has been reported that testicular RFRP and GPR147 are involved in steroidogenesis and spermatogenesis in male rats (Zhao *et al.* 2010; Anjum *et al.* 2014). Similarly, kisspeptin and its receptor also contribute to some testicular functions (Hsu *et al.* 2014). It remains unclear whether the LPS-induced changes in GPR147 expression observed in the present study contributed to the reductions in the rats' serum testosterone levels.

In summary, hypothalamic Kiss1r and RFRP mRNA expression and testicular GPR147 mRNA expression were altered by the injection of LPS in male rats. However, the roles of these alterations in the LPS-induced suppression of the HPG axis remain unclear.

ACKNOWLEDGEMENT

This work was supported by JSPS KAKENHI Grant Number 26462526.

REFERENCES

1 Anjum S, Krishna A, Tsutsui K (2014). Inhibitory roles of the mammalian GnIH ortholog RFRP3 in testicular activities in adult mice. J Endocrinol. **223**: 79–91.
2 Castellano JM, Bentsen AH, Romero M, Pineda R, Ruiz-Pino F, Garcia-Galiano D, *et al.* (2010). Acute inflammation reduces kisspeptin immunoreactivity at the arcuate nucleus and decreases responsiveness to kisspeptin independently of its anorectic effects. Am J Physiol Endocrinol Metab. **299**: 54–61.
3 Hsu MC, Wang JY, Lee YJ, Jong DS, Tsui KH, Chiu CH (2014). Kisspeptin modulates fertilization capacity of mouse spermatozoa. Reproduction. **147**: 835–845.
4 Iwasa T, Matsuzaki T, Murakami M, Kinouchi R, Ogata R, Kuwahara A, *et al.* (2009). Neonatal lipopolysaccharide exposure attenuates the homotypic stress-induced suppression of LH secretion in adulthood in male rat. Int J Dev Neurosci. **27**: 345–349.
5 Iwasa T, Matsuzaki T, Tungalagsuvd A, Munkhzaya M, Kawami T, Niki H, *et al.* (2014). Hypothalamic Kiss1and RFRP gene expressions are changed by a high dose of lipopolysaccharide in female rats. Horm Behav. **66**: 309–316.
6 Khan AR, Kauffman AS (2012). The role of kisspeptin and RFamide-related-3 neurons in the circadian-timed preovulatory luteinizing hormone surge. J Neuroendocrinol. **24**: 131–143.
7 Murakami M, Matsuzaki T, Iwasa T, Yasui T, Irahara M, Osugi T, *et al.* (2008). Hypophysiotropic role of RFamide-related peptide-3 in the inhibition of LH secretion in female rats. J Endocrinol. **199**: 105–112.
8 Zhao S, Zhu E, Yang C, Bentley GE, Tsutsui K, Kriegsfeld LJ, *et al.* (2010). RFamide-related peptide and messenger ribonucleic acid expression in mammalian testis: association with the spermatogenic cycle. Endocrinology. **151**: 617–627.