Songklanakarin J. Sci. Technol.
43 (4), 1018-1021, Jul. - Aug. 2021

http://www.sjst.psu.ac.th

Original Article

Changes of FSH and LH receptors expressions in rat testis after methamphetamine exposure

Paweena Kaewman, Sutisa Nudmamud-Thanoi, and Samur Thanoi*

Department of Anatomy and Centre of Excellence in Medical Biotechnology, Faculty of Medical Science, Naresuan University, Mueang, Phitsanulok, 65000 Thailand

Received: 22 August 2019; Revised: 21 June 2020; Accepted: 4 August 2020

Abstract

Methamphetamine (METH), the most widely used psychostimulant in Thailand, has been reported to have harmful effects on testicular functions and sperm quality, but its mechanisms are still unclear. Since follicle–stimulating hormone (FSH) and luteinizing hormone (LH) play important roles in spermatogenesis through their receptors in testis, we therefore focused on the changes in FSH and LH receptors after METH administration. Male Sprague–Dawley rats were divided into control and METH–administrated groups. The mRNA and protein expressions were measured using quantitative RT–PCR, and immunohistochemistry. The results showed that the rats in AB METH and ED–binge METH groups had significantly lower the FSH receptor protein expression (strong expression in the stages XII, XIII, XIV, and I of the spermatogenic cycle) compared with the control group but had no significant difference in the mRNA expression of FSH and LH receptors. These results may reflect the adverse effects of METH on FSH functions in testis.

Keywords: FSH receptor, LH receptor, methamphetamine, testis

1. Introduction

Methamphetamine (METH) is an addictive drug with powerful adverse effects on the central nervous system. The tablet form of METH, called Yaba, is the most popular illicit drug for the abusers in Thailand (Chomchai & Chomchai, 2015). Nowadays, it is very well known that METH use cause many serious risks to health. Previous studies have reported the adverse effects of METH including the induction of oxidative stress and the increase in the percentage of apoptotic tubules and testicular germ cells in the testis (Alavi, Taghavi, & Moallem, 2008; Nudmamud–Thanoi & Thanoi, 2011; Yamamoto *et al.*, 2002). METH can induce abnormal sperm morphology and the decrease of sperm concentration (Lin *et al.*, 2014; Nudmamud–Thanoi & Thanoi, 2011). Additionally, changes in testosterone and gonadotropin concentrations have been reported in METH–administrated animals (Fronczak, Kim, & Barqawi, 2012). The altered expression of sex steroid hormones receptors, progesterone, and estrogen receptors in the rat testis has been found after METH abuse (Nudmamud–Thanoi, Sueudom, Tangsrisakda, & Thanoi, 2016). Interestingly, based on Jin's review (Jin & Yang, 2014), the functions of the sex steroid hormones are mainly regulated by gonadotropins including follicle–stimulating hormone (FSH) and luteinizing hormone (LH). Conversely, these hormones can be the regulators of FSH and LH secretion.

FSH and LH are glycoprotein hormones that are produced and released from the anterior pituitary. The binding of FSH and LH with their receptors in the testis, FSH receptor (FSHR) and LH receptor (LHR), can promote the differentiation and proliferation of the testicular cells as well as steroidogenesis (O'Donnell, Stanton, & de Kretser, 2017). Moreover, there is evidence that these hormones and the sex steroid hormones have synergistic effects on the induction of spermatogenesis in the testis (Jin & Yang, 2014). These findings lead to the hypothesis that METH might affect not only the sex steroid hormones functions but also FSH and LH functions in the testis. Therefore, the purpose of the present study was to determine the changes in FSH and LH receptor expressions in the testis of METH–administrated rats.

*Corresponding author
Email address: samurt@nu.ac.th

2. Materials and Methods

2.1 Animals and METH administration

Animal management and METH administration protocol were approved by Naresuan University Animal Care and Use Committee, Thailand. The protocols were adapted from Segal *et al.* (Segal, Kuczenski, O'Neil, Melega, & Cho, 2003). Briefly, male Sprague–Dawley rats aging 5 weeks (200–250 g) were divided into four groups. The rats in a control group (n = 10) were injected intraperitoneally (i.p.) with 0.9% normal saline for 15 days. In the acute binge group (AB METH, n = 10), the rats were injected (i.p.) with 0.9% normal saline for 14 days followed by a binge dose of METH, 6 mg/kg METH 4 times a day at 2 h intervals, on day 15. During day1–14 of the experiment, the rats in the escalating dose (ED METH, n = 9) and escalating dose–binge (ED–binge METH, n = 10) groups were injected (i.p.) with an escalating dose of METH, gradually increasing doses of METH 0.1–4 mg/kg. Then, on day 15, those rats were injected (i.p.) with 0.9% normal saline and the binge dose of METH for the ED METH and ED–binge METH groups, respectively. At the end of the treatment, testes were removed immediately after sacrifice. Then, the left testis was fixed in 10% natural paraformaldehyde while the right testis was frozen at –80°C.

2.2 Reverse transcription polymerase chain reaction (RT–PCR) analysis

Total RNA was extracted from the frozen testis using Trizol reagent (Invitrogen, CA) and chloroform. Then, cDNA was synthesized using the iScript cDNA Synthesis kit (Bio–Rad, CA) according to the manufacturer's protocol. Table 1 shows the oligonucleotide primers of FSHR, LHR, and GAPDH (internal control) genes that were used in this study. FSHR and GAPDH primers were employed according to Romero *et al.* (Romero, Paredes, Dissen, & Ojeda, 2002) and Barlow *et al.* (Barlow *et al.*, 2003), respectively, while LHR primer was designed by using Primer3 software (freely available at http://bioinfo.ut.ee/primer3-0.4.0/) and the Oligo Analyzer software version 1.0.3 (Freeware, Teemu Kuulasmaa, Finland). To check the specificity of these primers, the complementary with other genes in the rat genome was done using the NCBI "Primer–BLAST" tool. The PCR sequences were amplified using Ssofast™ EvaGreen supermix and performed on a CFX96 Touch™ Real–Time PCR system (Bio–Rad, CA). The expression levels of FSHR and LHR genes were represented as relative mRNA expression values by the normalization with GAPDH gene.

2.3 Immunohistochemistry analysis

The left testis was dehydrated with serial ethanol and then embedded in paraffin. 5–µm–thick paraffin sections were prepared. In this study, FSHR immunostaining was performed with rabbit anti–FSHR polyclonal antibody (sc–13935; Santa Cruz, CA) as a primary antibody. The sections were incubated with biotinylated secondary antibody and then the immunosignal enhanced using the avidin–biotinylated horseradish peroxidase complexes (ABC kit) (Vector, CA). The FSHR immunoreactivity was visualized using DAB (3,3'–Diaminobenzidine) (Vector, CA) as a chromogen for immunohistochemistry. After that, the sections were dehydrated with serial ethanol and mounted with mounting media. The FSHR expression in each stage of the spermatogenic cycle that was classified according to Thanoi *et al.* (Thanoi, Janphet, & Nudmamud–Thanoi, 2020), was determined. The intensity was measured using an NIH ImageJ software (freely available at http://imagej.nih.gov/ij/download.html). Finally, the expression levels of FSHR protein were determined as the relative optical density (ROD) values.

Table 1. Sequence of oligonucleotide primers

Genes	Forward primer (5'–3')	Reverse primer (5'–3')
FSHR	CATCACTGTGTCCAAGGCCA	TGCGGAAGTTCTTGGTGAAAA
LHR	GTTCACCCAAGACACTCCAATG	TCAGCCAAATCAGGACCCTA
GAPDH	AATGTATCCGTTGTGGATCTGA	GCCTGCTTCACCACCTTCT

2.4 Statistical analysis

The data were represented as mean ± SEM. The differences between groups were analyzed using one–way ANOVA followed by Dunnett's post hoc test. Statistical significance was considered at $p < 0.05$.

3. Results and Discussion

The mRNA expressions of FSHR and LHR were decreased in METH–administrated groups compared to the control group, but those changes did not reach statistical significance (Figure 1 and Figure 2).

The results of immunohistochemistry indicated the strong expression of FSHR protein on Sertoli cells, and testicular germ cells in the stages XII, XIII, XIV, and I of the spermatogenic cycle in the rat testis. In the stages XII and XIII, the FSHR expression was found in various cell types within the seminiferous tubules consisting of Sertoli cells, spermatogonia, zygotene spermatocytes, and elongated spermatids but not pachytene, or diplotene spermatocytes (Figures 3A and 3B). There was FSHR expression on Sertoli cells, spermatogonia, and elongated spermatids in the stages XIV and I of the spermatogenic cycle, see Figure 3C and 3D. Besides, the FSHR protein also expressed on secondary spermatocytes and round spermatids in the stages XIV and I, respectively. Interestingly, the FSHR expression on pachytene spermatocyte was detected in the stages XIV and I but was not found in stage XII. The levels of the FSHR protein expression in the rat testis were analyzed in the stages XII, XIII, XIV, and I of the spermatogenic cycle. Figure 4 shows that the levels of ROD in binge METH–administrated groups, AB METH (0.227 ± 0.011), and ED–binge METH (0.233 ± 0.007) groups, were significantly decreased ($p = 0.011$ and $p = 0.028$) when compared to the control group (0.267 ± 0.004), whereas the decrease of ROD levels in ED METH group (0.244 ± 0.009) did not reach statistical significance.

The results of FSHR protein localization in this study are consistent with a previous study which reported the localization of FSHR protein on the Sertoli cells and testicular

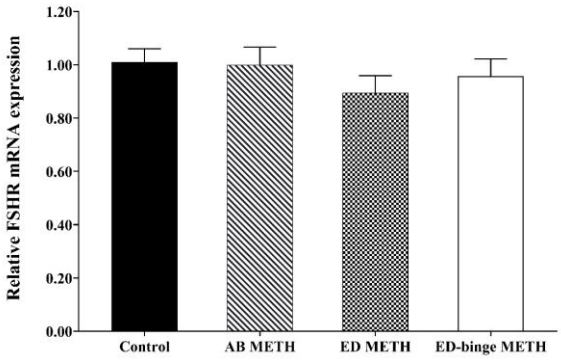

Figure 1. The relative FSHR mRNA expression in testis of METH–administrated rats compared to the control group. Values are mean ± SEM, n = 9–10 per group.

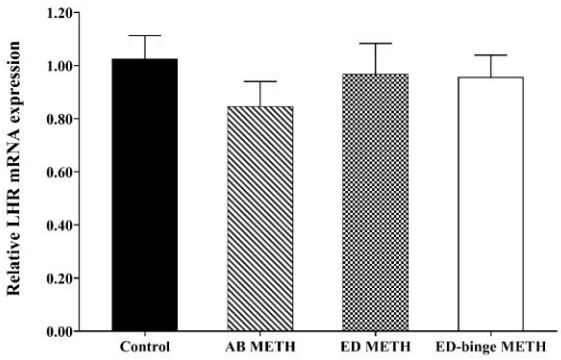

Figure 2. The relative LHR mRNA expression in testis of METH–administrated rats compared to the control group. Values are mean ± SEM, n = 9–10 per group.

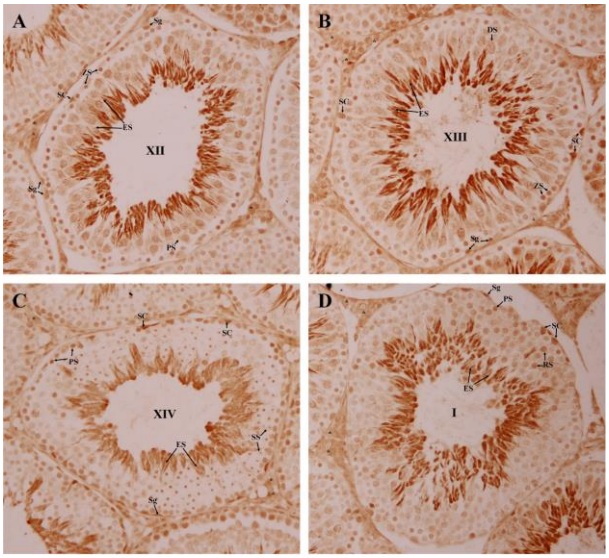

Figure 3. Illustrating the FSHR protein expression in the stages XII (A), XIII (B), XIV(C) and I (D) of the spermatogenic cycle in rat testis. Sertoli cells (SC), Leydig cells (asterisks), as well as testicular germ cells including spermatogonia (Sg), zygotene spermatocytes (ZS), pachytene spermatocytes (PS), secondary spermatocytes (SS), diplotene sperma tocytes (DS), round spermatids (RS) and, elongated spermatids (ES)

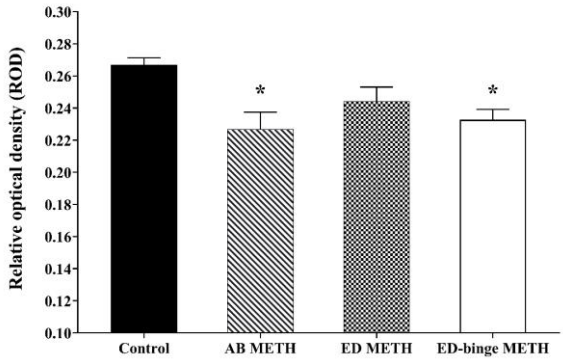

Figure 4. The relative FSHR protein expression in testis of METH–administrated rats compared to the control group. Values are mean ± SEM, n = 4–5 per group (*p < 0.05; Dunnett's post hoc test).

germ cells (Baccetti *et al.*, 1998). Moreover, it has been reported that the levels of FSHR expression were different in each stage of the spermatogenic cycle (14 stages I–XIV) in the rat. In agreement with our finding, the highest levels of FSHR expression have been found in the stages XIII, XIV, and I (Heckert & Griswold, 1991; Simoni, Gromoll, & Nieschlag, 1997). Furthermore, the present study can provide more information about the localization of FSHR protein on the testicular germ cells in each stage of the spermatogenic cycle, especially the stages XII, XIII, XIV, and I.

Our results indicated that METH may affect the FSH and LH functions in the testis through the reduction of their receptors. METH administration slightly disturbed the transcription of FSH and LH receptors in the testis. The binding of LH to its receptor induces the testosterone production by Leydig cells in the testis. There is evidence that the loss of LHR functions in mice resulted in the decrease of testosterone levels (Lei *et al.*, 2001; Zhang, Pakarainen, Poutanen, Toppari, & Huhtaniemi, 2003). Thus, the decrease of LHR expression might be one reason why the testosterone levels were decreased after METH administration. Interestingly, the translation of FSHR was strongly affected by METH. Because FSH plays a role to prevent the apoptosis of testicular germ cells, the decrease of FSH functions might cause an increase of apoptosis in the testis after METH exposure. Additionally, the attenuation of FSH functions to control the differentiation and proliferation of the testicular cells can cause poor sperm quality (Sofikitis *et al.*, 2008). Studying in FSHR knockout mice have shown that the aberration of FSHR resulted in the reduction of sperm production and quality (Dierich *et al.*, 1998; O'Shaughnessy, Monteiro, & Abel, 2012).

4. Conclusions

In summary, our findings can provide the effect of METH on FSH and LH functions in the testis. The decrease of FSH and LH receptors expression refers to the impairment of their functions which play an important role in testicular functions and sperm quality. In particular, the result of the FSHR protein expression indicated that METH has a strong effect to attenuate the FSH functions in the testis. Finally, these findings will be useful to understand the mechanism of METH in the testis.

Acknowledgements

We appreciate Thailand Research Fund (TRF) and Naresuan University for the financial and facilities support. PK was supported by the Royal Golden Jubilee Ph.D. Program, Thailand.

References

Alavi, S. H., Taghavi, M. M., & Moallem, S. A. (2008). Evaluation of effects of methamphetamine repeated dosing on proliferation and apoptosis of rat germ cells. *Systems Biology in Reproductive Medicine, 54*(2), 85-91. doi:10.1080/19396360801952078

Baccetti, B., Collodel, G., Costantino-Ceccarini, E., Eshkol, A., Gambera, L., Moretti, E., . . . Piomboni, P. (1998). Localization of human follicle-stimulating hormone in the testis. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology, 12*(11), 1045-1054. doi:10.1096/fasebj.12.11.1045

Barlow, N. J., Phillips, S. L., Wallace, D. G., Sar, M., Gaido, K. W., & Foster, P. M. (2003). Quantitative changes in gene expression in fetal rat testes following exposure to di(n-butyl) phthalate. *Toxicological sciences, 73*(2), 431-441. doi:10.1093/toxsci/kfg087

Chomchai, C., & Chomchai, S. (2015). Global patterns of methamphetamine use. *Current Opinion in Psychiatry, 28*(4), 269-274. doi:10.1097/yco.0000000000000168

Dierich, A., Sairam, M. R., Monaco, L., Fimia, G. M., Gansmuller, A., LeMeur, M., & Sassone-Corsi, P. (1998). Impairing follicle-stimulating hormone (FSH) signaling in vivo: targeted disruption of the FSH receptor leads to aberrant gametogenesis and hormonal imbalance. *Proceedings of the National Academy of Sciences of the United States of America, 95*(23), 13612-13617. doi:10.1073/pnas.95.23.13612

Fronczak, C. M., Kim, E. D., & Barqawi, A. B. (2012). The insults of illicit drug use on male fertility. *Journal of andrology, 33*(4), 515-528. doi:10.2164/jandrol.110.011874

Heckert, L. L., & Griswold, M. D. (1991). Expression of follicle-stimulating hormone receptor mRNA in rat testes and Sertoli cells. *Molecular Endocrinology, 5*(5), 670-677. doi:10.1210/mend-5-5-670

Jin, J. M., & Yang, W. X. (2014). Molecular regulation of hypothalamus-pituitary-gonads axis in males. *Gene, 551*(1), 15-25. doi:10.1016/j.gene.2014.08.048

Lei, Z. M., Mishra, S., Zou, W., Xu, B., Foltz, M., Li, X., & Rao, C. V. (2001). Targeted disruption of luteinizing hormone/human chorionic gonadotropin receptor gene. *Molecular Endocrinology, 15*(1), 184-200. doi:10.1210/mend.15.1.0586

Lin, J. F., Lin, Y. H., Liao, P. C., Lin, Y. C., Tsai, T. F., Chou, K. Y., . . . Hwang, T. I. (2014). Induction of testi cular damage by daily methamphetamine administra tion in rats. *The Chinese Journal of Physiology, 57*(1), 19-30. doi:10.4077/cjp.2014.bab 155

Nudmamud-Thanoi, S., Sueudom, W., Tangsrisakda, N., & Thanoi, S. (2016). Changes of sperm quality and hormone receptors in the rat testis after exposure to methamphetamine. *Drug and Chemical Toxicology, 39*(4), 432-438. doi:10.3109/01480545.2016.1141421

Nudmamud-Thanoi, S., & Thanoi, S. (2011). Metham phetamine induces abnormal sperm morphology, low sperm concentration and apoptosis in the testis of male rats. *Andrologia, 43*(4), 278-282. doi:10.1111/j.1439-0272.2010.01071.x

O'Donnell, L., Stanton, P., & de Kretser, D. M. (2017). Endocrinology of the male reproductive system and spermatogenesis. In K. R. Feingold , B. Anawalt, & A. Boyce (Eds.), *Endocrinology of male reproduction* (pp. 1-69). South Dartmouth, MA: Endotext. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK279031/

O'Shaughnessy, P. J., Monteiro, A., & Abel, M. (2012). Testicular development in mice lacking receptors for follicle stimulating hormone and androgen. *PloS one, 7*(4), e35136. doi:10.1371/journal.pone.0035136

Romero, C., Paredes, A., Dissen, G. A., & Ojeda, S. R. (2002). Nerve growth factor induces the expression of functional FSH receptors in newly formed follicles of the rat ovary. *Endocrinology, 143*(4), 1485-1494. doi:10.1210/endo.143.4.8711

Segal, D. S., Kuczenski, R., O'Neil, M. L., Melega, W. P., & Cho, A. K. (2003). Escalating dose metham phetamine pretreatment alters the behavioral and neurochemical profiles associated with exposure to a high-dose methamphetamine binge. *Neuro psychopharmacology, 28*(10), 1730-1740. doi:10.1038/sj.npp.1300247

Simoni, M., Gromoll, J., & Nieschlag, E. (1997). The follicle-stimulating hormone receptor: Biochemistry, molecular biology, physiology, and pathophysio logy. *Endocrine Reviews, 18*(6), 739-773. doi:10.1210/edrv.18.6.0320

Sofikitis, N., Giotitsas, N., Tsounapi, P., Baltogiannis, D., Giannakis, D., & Pardalidis, N. (2008). Hormonal regulation of spermatogenesis and spermiogenesis. *The Journal of Steroid Biochemistry and Molecular Biology, 109*(3-5), 323-330. doi:10.1016/j.jsbmb.2008.03.004

Thanoi, S., Janphet, S., & Nudmamud-Thanoi, S. (2019). Changes of dopamine D2, alpha1 adrenergic receptor expressions and developmental stages of seminiferous tubule in rat testis after metham phetamine administration. *Songklanakarin Journal of Science and Technology, 42*(4), 928-934.

Yamamoto, Y., Yamamoto, K., Hayase, T., Abiru, H., Shiota, K., & Mori, C. (2002). Methamphetamine induces apoptosis in seminiferous tubules in male mice testis. *Toxicology and Applied pharmacology, 178*(3), 155-160. doi:10.1006/taap.2001.9330

Zhang, F. P., Pakarainen, T., Poutanen, M., Toppari, J., & Huhtaniemi, I. (2003). The low gonadotropin-independent constitutive production of testicular testosterone is sufficient to maintain spermato genesis. *Proceedings of the National Academy of Sciences of the United States of America, 100*(23), 13692-13697. doi:10.1073/pnas.2232815100