Pakistan J. Zool., vol. 49(1), pp 379-382, 2017. **DOI**: http://dx.doi.org/10.17582/journal.pjz/2017.49.1.sc2

Short Communication

Effect of Variable Kisspeptin Doses on Prostatic Citric Acid Levels in Male Mice

Faiqah Ramzan*[1] and Muhammad Haris Ramzan[2]

[1]*Gomal Centre of Biochemistry and Biotechnology, Gomal University, Dera Ismail Khan, Khyber Pakhtukhwa, Pakistan*

[2]*Department of Physiology, Khyber Medical University Institute of Medical Sciences, Kohat, Khyber Pakhtunkhwa, Pakistan*

ABSTRACT

Kisspeptin, a product of the KISS1 gene, plays an essential role in the regulation of spermatogenesis acting primarily at the hypothalamic level of the gonadotropic axis. Citric acid is synthesized and secreted by the prostate gland. Its synthesis is regulated by androgens. As, dose dependent degeneration of prostate gland has been described following intraperitoneal kisspeptin treatment. However, effects of kisspeptin administration on the levels of prostatic citric acid remain elusive till date. The present study, therefore, addresses the effects of 12 day administration of kisspeptin on prostatic citric acid levels in male mice. Kisspeptin-10 was administered intraperitoneally in different dosage concentrations (1µg, 1 ng, and 10 ρg) to adult male mice twice daily for 12 days. Prostatic citric acid levels were determined photometrically. The levels decreased significantly at all tested doses. Intermittent administration of Kisspeptin –10 negatively regulates the prostatic citric acid levels in adult male mice.

Article Information
Received 16 December 2015
Revised 28 September 2016
Accepted 10 October 2016
Available online 28 November 2016

Authors' Contributions
FR designed the study and conducted the entire work. MHR and FR wrote the article.

Key words
Kisspeptin, Prostate gland, Citric acid levels.

The framework supporting the gonadotropic axis is composed of three major elements: hypothalamic gonadotropin-releasing hormone (GnRH), pituitary gonadotropins (Luteinizing Hormone, LH; and Follicle Stimulating Hormone, FSH) and gonadal sex steroids (Pierantoni *et al*., 2002). A plethora of central and peripheral signals, modulates the gonadotropic axis (Fink, 2000). The kisspeptins has been identified as novel multifunctional peptides having ability to stimulate GnRH/LH/FSH secretion through the activation of GPR54, a classical G protein coupled receptor, formerly known as AXOR12 or OT7T175 (Shahab *et al*., 2005; Thompson *et al*., 2004). Kisspeptins, the products of Kiss-1 gene, are neuropeptides belonging to the family of RF-amide peptides (Ukena and Tsutsui, 2005); are encoded as a 145-amino acid product, Kp-145. Kp -145 is cleaved proteolitically into shorter peptides (Kp-54, -10, -13 and -14). Kp-54 and its cleavage products share a common RF-amide C-terminal decapeptide and exhibit the same affinity and efficacy for GPR54 (Kotani *et al*., 2001).

The existence of significant concentrations of citric acid in mammalian reproductive organs was first reported by Schersten (1929). Citric acid is a major component of semen, and the concentration is particularly high in bull semen where it may exceed 1%. It is usually absent from epididymal semen but is present in ejaculated and sometimes also in ampullar semen (Humphrey and Mann, 1949). In man, Schersten (1929) found high concentrations of citric acid of prostatic origin in seminal plasma. Human prostatic tissue is rich in citric acid (Barron and Huggins, 1946a, b). Citric acid has been associated with the gelification, coagulation and liquefaction of semen in rats (Hart, 1970), monkeys (Hoskins and Patterson, 1967), and humans (Huggins and Neal, 1942).

In a previous study, we have demonstrated that continuous kisspeptin exposure causes degeneration of prostate gland as evidenced by increased tubular lumen and decrease in epithelial height and epithelial folds in the mucosa (Ramzan *et al*., 2012). This might have compromised the secretory activity of prostate gland. Chemical determinations of citric acid in human semen provide a simple and convenient means for a quantitative assessment of the secretory function of the prostate gland (Marberger *et al*., 1962). The present study measures the prostatic citric acid levels in adult mice after administration of a range of kisspeptin doses to evaluate the function of prostate gland after kisspeptin exposure.

Methods and materials

Animals and maintenance

Forty (n=40), adult male albino mice (*Mus musculus*) of Swiss strain with an average weight of 50±5g were

* Corresponding author: faiqah_ramzan@yahoo.com
0030-9923/2017/0001-0379 $ 9.00/0

purchased from the National Institute of Health, Islamabad and maintained in the animal house facility of Gomal University. Ten mice were housed per cage (15″ × 11″ × 9″, steel mesh cages) under standard conditions of 12L:12D h photoperiod, 25±2ºC temperature controlled with automatic timers and adjustable controls for heating and cooling. Standard rat diet and water were provided *ad libitum*.

All animal handling and subsequent sacrifice was carried out according to the guidelines provided by the "Institutional Review Board" of the Gomal Centre of Biochemistry and Biotechnology, Gomal University, Dera Ismail Khan for humane use of animals for scientific research. Animal handling was also in accordance with European Union guidelines for the use of laboratory animals.

Experimental procedures

Kisspeptin (metastin 45-54 or kisspeptin-10; 1mg lyophilized powder) was purchased from Calbiochem (EMD Biosciences, Inc. La Jolla, CA) and was dissolved in 1 ml Dimethylesulphoxide (DMSO) to give a stock solution of 1 mg ml^{-1} that was diluted further with distilled water (dH_2O) and was administered intraperitonealy (i.p.).

Animals were randomly assigned to four groups (n =10 in each). Group I mice constituted control and received 0.9% w/v physiological saline, (DMSO was added to saline at the same concentration as it was added to kisspeptin stock and was further diluted to concentration equivalent to the experimental doses), group-II mice received 10 ρg, group-III received 1 ng and group IV received 1µg kisspeptin as twice daily after every 12 h for 12 days. Kisspeptin doses were selected as previously described (Ramzan and Qureshi, 2011). Three h after the last dose of the peptide, animals were anesthetized with sodium pentobarbital (60 mg kg^{-1} b.w. i.p.). Prostate glands were dissected out, weighed, rinsed in phosphate buffered saline and stored at -50°C until assayed. To determine the concentrations of fructose in prostate glands,1 ml of distilled water was added to the tissue samples, macerated (Gonzales, 1989) and then centrifuged at 5000 rpm for 10 min. Supernatant was aspirated and levels of citric acid were determined photometrically.

Citric acid estimation

Seminal fructose was estimated photometrically. Citric acid levels were estimated using citric acid test kit (FertiPro N.V.IndustrieparkNoord 32, 8730 Beernem – Belgium). Assays were carried out according to the manufacturer's instructions. The absorbance was measured at 505 nm.

Results were expressed as mean ± SD. The results obtained, were analyzed and compared by one way ANOVA followed by post hoc Tukey's adjustment using the Statistical Package for Social Sciences (SPSS, version 16, Inc, Chicago, Illinois, USA). $P< 0.05$ was considered to be statistically significant.

Results and discussion

Figure 1 shows effect of kisspeptin an total body weight, prostatic weight and citric acid level of prostate gland of male mice. There is no significant difference in the body and prostatic weight, although the concentration of citric acid was significantly reduced ($P<0.001$) in the treated groups when compared with the control group.

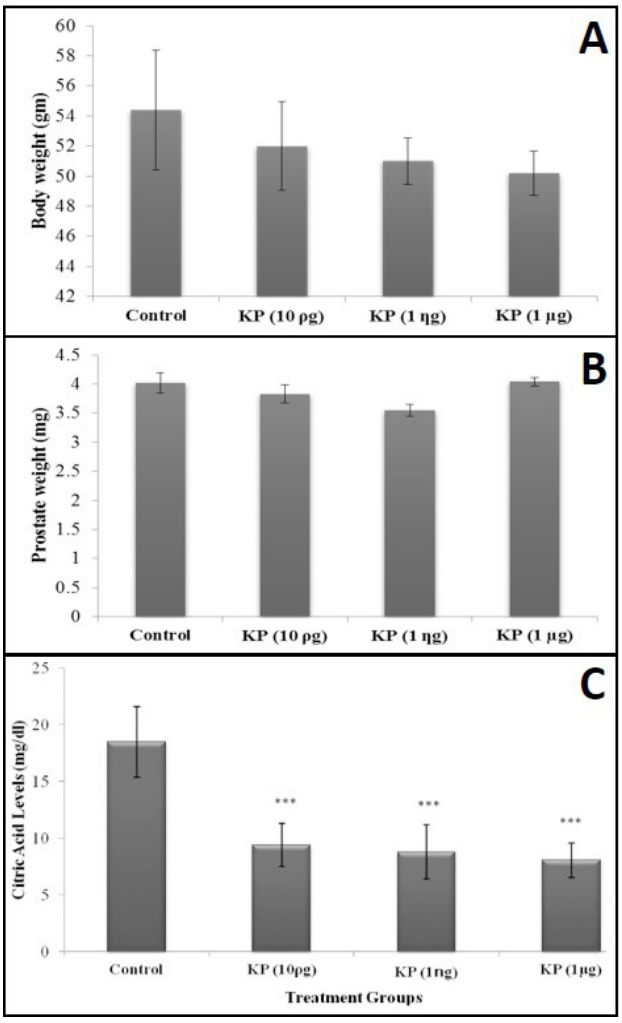

Fig. 1. Effect of different concentrations of KP on body weight (A), prostatic weight (B) and prostatic citric acid levels (mg/dl) (C) in male mice. Values are expressed as mean±SD.

The kisspeptin/GPR54 interaction has finally been identified in recent years as key regulator of the HPG axis (de Roux *et al*., 2003; Seminara *et al*., 2003). Although, the acute effects of central and peripheral administration of kisspeptin in rats, mice (Gottsch *et al*., 2004; Navarro *et al*., 2004, 2005) and broiler breeder birds (Wahab *et al*., 2012), as well as effects of long term kisspeptin administration on structure and functional aspects of testicular tissue in prepubertal (Ramzan and Qureshi, 2011) and adult testes (Thompson *et al*., 2004, 2009) have been explored in detail. Moreover, the effects of kisspeptin administration on histomorphological and ultrastructure of accessory sex glands, the seminal vesicle and prostate have also been documented (Ramzan *et al*., 2012, 2013). The seminal fructose levels following kisspeptin challenge in male mice have also been investigated (Ramzan *et al*., 2014). However, to date there is no report on kisspeptin effects on the functional aspects of prostate gland. The present study shows that the sub chronic 12 days i.p. kisspeptin administration at 1µg, 1*n*g and 10 ρg dose led to a significant decrease in prostatic citric acid levels in male mice.

Prostatic citrate production is androgen dependent, since castration and hypophysectomy results in rapid loss of prostatic citrate and seminal fructose. The administration of physiologic amounts of androgen restores prostatic citrate and seminal fructose values to normal levels (Humphrey and Mann, 1949; Mann and Parsons, 1947). The activity of seminal vesicle and prostate is known to be under the control of testosterone produced by the testis. Lindner and Mann (1960) described a significant correlation between the testosterone content of bull testes and the weight of seminal vesicles, their fructose and citric acid contents. Similar correlations between the action of administered testosterone and the level of acid phosphatase and citric acid in semen have been observed by various workers (Mann, 1964).

Fawcett (1986) also proved that testosterone is essential for the maintenance of height of the mucosal epithelium required for productions of continuous prostate secretions (Fawcett, 1986). The growth and active secretion of accessory reproductive tissues are dependent on the presence of circulating androgens (Higgins *et al*., 1976a, b; Brandes, 1974). The prostatic epithelial height is known to be androgen-dependent (Gonzales *et al*., 2005) and secretory activity by the prostate and seminal vesicles therefore is a sensitive, androgen dependent function (Veneziale *et al*., 1977).

In benign prostatic hypertrophy citrate accumulation is striking while in early and advanced prostatic carcinoma there was a striking straight line reduction in citrate concentration directly related to size and extent of carcinoma. In advanced carcinoma the citrate values were less than 10% of those found in benign hypertrophy (Cooper and Farid, 1963).

Conclusion

The study reveals that chronic kisspeptin-10 administration down regulates the citric acid content of the prostate gland in adult male mice.

Acknowledgments

The study was funded by the Startup Research Grant provided to the corresponding author by the Higher Education Commission (HEC), H-9, Islamabad, Pakistan. The authors are indebted to Dr. Muhammad Aslam Khan, Clinical Pathologist, Department of Pathology, District Headquarter Teaching Hospital, Dera Ismail Khan KPK, Pakistan for technical assistance.

Conflict of interest statement

We declare that we have no conflict of interest.

References

Barron, E. S. and Huggins, C., 1946a. *Proc. Soc. exp. Biol. Med.*, **62**: 195. http://dx.doi.org/10.3181/00379727-62-15418

Barron, E. S. and Huggins, C., 1946b. *J. Urol.*, **55**: 385-390.

Brandes, D., 1974. In: *Male accessory sex organs. Structure and function in mammals* (ed. D. Brandes), Academic Press, New York.

Cooper, J. F. and Farid, I., 1963. *J. Surg. Res.*, **3**: 112-121. http://dx.doi.org/10.1016/S0022-4804(63)80041-4

De Roux, N., Genin, E., Carel, J. C., Matsuda, F., Chaussain, J. L. and Milgrom, E., 2003. *Proc. natl. Acad. Sci. U. S. A.*, **100**: 10972-10976. http://dx.doi.org/10.1073/pnas.1834399100

Fawcett, D. W., 1986. In: *A textbook of histology* (eds. W. Bloom and D. W. Fawcett) W.B. Saunders Company, Philadelphia.

Fink, G., 2000. In: *Neuroendocrinology in physiology and medicine* (eds. P. M. Conn and M. E. Freeman). Humana Press Inc, New Jersey.

Gonzales, G. F., 1989. *Arch. Androl.*, **22**: 1-13. http://dx.doi.org/10.3109/01485018908986745

Gonzales, G. F., Miranda, S., Nieto, J., Fernandez, G., Yucra, S., Rubio, J., Yi, P. and Gasco, M., 2005. *Reprod. Biol. Endocrinol.*, **3**: 5. http://dx.doi.org/10.1186/1477-7827-3-5

Gottsch, M. L., Cunningham, M. J., Smith, J. T., Popa, S. M., Acohido, B. V., Crowley, W. F., Seminara, S., Clifton, D. K. and Steiner, R. A., 2004. *Endocrinology*, **145**: 4073-4077. http://dx.doi.

org/10.1210/en.2004-0431
Hart, R. G., 1970. *Biol. Reprod.*, **3,** 347-352.
Higgins, S. J., Burchell, J. M. and Minwaring, W. I., 1976a. *Biochem. J.*, **158:** 271-282. http://dx.doi.org/10.1042/bj1580271
Higgins, S. J., Burchell, J. M. and Mainwaring, W. I., 1976b. *Biochem. J.*, **160**: 43-48. http://dx.doi.org/10.1042/bj1600043
Hoskins, D. D. and Patterson, D. L., 1967. *J. Reprod. Fertil.*, **13**: 337-340. http://dx.doi.org/10.1530/jrf.0.0130337
Huggins, C. and Neal, W., 1942. *J. exp. Med.*, **76**: 527-541. http://dx.doi.org/10.1084/jem.76.6.527
Humphrey, G. F. and Mann, T., 1949. *Biochem. J.*, **44**: 97-105. http://dx.doi.org/10.1042/bj0440097
Kotani, M., Detheux, M., Vandenbogaerde, A., Communi, D., Vanderwinden, J. M., Lepoul, E., Brezillon, S., Tyldesly, R., Suarez-Huerta, N., Vandeput, F., Blanpain, C., Schiffmann, S. N., Vassart, G. and Parmentier, M., 2001. *J. biol. Chem.*, **276**: 34631-34636. http://dx.doi.org/10.1074/jbc.M104847200
Linder, H. R. and Mann, T., 1960. *J. Endocrinol.*, **21**: 341-360.
Mann, T., 1964. *The biochemistry of semen and of the male reproductive tract*, Wiley, New York.
Mann, T. and Parsons, U., 1947. *Nature*, **160**: 294. http://dx.doi.org/10.1038/160294a0
Marberger, H., Marberger, E., Mann, T. and Lutwak-Mann, C., 1962. *Br. med. J.*, **1**: 835-836. http://dx.doi.org/10.1136/bmj.1.5281.835
Navarro, V. M., Castellano, J. M., Fernandez-Fernandez, R., Tovar, S., Roa, J., Mayen, A., Nogueiras, R., Vazquez, M. J., Barreiro, M. L., Magni, P., Aguilar, E., Dieguez, C., Pinilla, L. and Tena-Sempere, M., 2005. *Endocrinology*, **146**: 156-163. http://dx.doi.org/10.1210/en.2004-0836
Navarro, V. M., Fernandez-Fernandez, R., Castellano, J. M., Roa, J., Mayen, A., Barreiro, M. L., Gaytan, F., Aguilar, E., Pinilla, L., Dieguez, C. and Tena-Sempere, M., 2004. *J. Physiol.*, **561**: 379-386. http://dx.doi.org/10.1113/jphysiol.2004.072298
Pierantoni, R., Cobellis, G., Meccariello, R. and Fasano, S., 2002. *Int. Rev. Cytol.*, **218**: 69-141. http://dx.doi.org/10.1016/S0074-7696(02)18012-0
Ramzan, F., Khan, M. A. and Ramzan, M. H., 2014. *Endocrine*, **45**: 144-147. http://dx.doi.org/10.1007/s12020-013-0016-x
Ramzan, F. and Qureshi, I. Z., 2011. *Life Sci.*, **88**: 246-256. http://dx.doi.org/10.1016/j.lfs.2010.11.019
Ramzan, F., Qureshi, I. Z., Ramzan, M. and Ramzan, M. H., 2012. *Reprod. Biol. Endocrinol.*, **10**: 18. http://dx.doi.org/10.1186/1477-7827-10-18
Ramzan, F., Qureshi, I. Z., Ramzan, M. and Ramzan, M. H., 2013. *Prostate*, **73**: 690-699. http://dx.doi.org/10.1002/pros.22609
Schersten, B., 1929. *Skand. Arch. Physiol.*, **58**: 90. http://dx.doi.org/10.1111/j.1748-1716.1930.tb00919.x
Seminara, S. B., Messager, S., Chatzidaki, E. E., Thresher, R. R., Acierno, J. S., JR., Shagoury, J. K., Bo-Abbas, Y., Kuohung, W., Schwinof, K. M., Hensrick, A. G., Zahn, D., Dixon, J., Kaiser, U. B., Slaugenhaupt, S. A., Gusella, J. F., O'Rahilly, S., Carlton, M. B., Crowley, Jr., W. F., Aparicio, S. A. and Colledge, W. H., 2003. *N. Engl. J. Med.*, **349**: 1614-1627. http://dx.doi.org/10.1056/NEJMoa035322
Shahab, M., Mastronardi, C., Seminara, S. B., Crowley, W. F., Ojeda, S. R. and Plant, T. M., 2005. *Proc. natl. Acad. Sci. U. S. A.,* **102**: 2129-2134. http://dx.doi.org/10.1073/pnas.0409822102
Thompson, E. L., Amber, V., Stamp, G. W., Patterson, M., Curtis, A. E., Cooke, J. H., Appleby, G. F., Dhillo, W. S., Ghatei, M. A., Bloom, S. R. and Murphy, K. G., 2009. *Br. J. Pharmacol.*, **156**: 609-625. http://dx.doi.org/10.1111/j.1476-5381.2008.00061.x
Thompson, E. L., Patterson, M., Murphy, K. G., Smith, K. L., Dhillo, W. S., Todd, J. F., Ghatei, M. A. and Bloom, S. R., 2004. *J. Neuroendocrinol.*, **16**: 850-858. http://dx.doi.org/10.1111/j.1365-2826.2004.01240.x
Ukena, K. and Tsutsui, K., 2005. *Mass Spectrom. Rev.*, **24**: 469-486. http://dx.doi.org/10.1002/mas.20031
Veneziale, C. M., Burns, J. M., Lewis, J. C. and Bucchi, K. A., 1977. *Biochem. J.*, **166**: 167-173. http://dx.doi.org/10.1042/bj1660167
Wahab, F., Riaz, T., Khan, L. A., Leprince, J., Vaudry, H. and Sempere, M.T., 2012. *Pakistan J. Zool.* **44**:7-14.