JOURNAL OF
INTEGRATED FIELD SCIENCE
Field Science Center, Graduate School of Agricultural Science, Tohoku University

Symposium mini review

Receptors in Spermatozoa – Their expressions and Functions

Yuki HIRADATE, Kenshiro HARA and Kentaro TANEMURA

Laboratory of Animal Reproduction and Development, Graduate School of Tohoku University,
468-1 Aramaki Aza Aoba, Sendai, Miyagi Japan

Keywords
spermatozoa, neurotensin, acrosome reaction, preimplantation embryo

Corresponding Author
Yuki HIRADATE,
yuki.hiradate.d4@tohoku.ac.jp

Abstract

For stable breeding of livestock, it is necessary to deepen the understanding of sperm having fertility. It is well known that spermatozoa have receptor patterns which are similar to those of nerve cells. Neurotensin (NTS) is initially isolated from hypothalamus, later its multiple functions in several tissues have been elucidated. However, there is still little information about the effects of NTS on the reproductive organs. This paper reviews the mechanism of ligand secretion from the female side as well as the expression of NTS receptors in spermatozoa and their physiological functions. It also describes the contribution of NTS to preimplantation embryo development and focuses on the function of NTS as a cofactor from fertilization to early embryonic development.

Introduction

Neurotensin (NTS), consisting of 13 amino acids, was first isolated from the bovine hypothalamus (Carraway *et al*., 1973) as inducing vasodilatory effect. NTS is processed from its precursor, pro-NTS by protein convertase (Kitabgi, 2006). The action of NTS has a wide variety of biological actions due to the difference in its localization (Carraway *et al*., 1977), including control of fat absorption (Li *et al*., 2016). In reproductive system, a former work reported its NTS induced contractions of smooth muscle, showed a possibility of assisting embryo transport (Reinecke, 1987). However, there is still limited information.

Expression patterns of NTS and NTS receptors and their effects on sperm function

For the first time, we have elucidated the expression and function of NTS and its receptors in a fertilized environment (Hiradate *et al*., 2014). We firstly determined NTS expression patterns using an antibody. The organizational structure of fallopian tube, where fertilization occurs, can be divided into an ampulla and an isthmus part. Spermatozoa pass from the uterus through the lumen and wait in the isthmus for ovulation. The ampulla is the place where the oocytes, which are ovulated from an ovary, stay, and also the place where fertilization occurs. The fact that immunoreactivity of the epithelial cells on both parts were immunostained suggests that NTS is secreted into the lumen. Furthermore, in bovine oviductal epithelial culture model, follicular fluid exposure upregulates *Nts* expression (Hasan *et al*., 2020). These evidence suggest NTS is one of the promising factors that promotes spermatozoa fertility.

Cumulus cells, cell layers surrounding the oocyte, have important functions for fertilization. Cumulus cells are also a source of secretion factors affecting sperm physiological functions as well as epithelial cells. To examine NTS mRNA expression in cumulus cells, qPCR was performed. PMSG following hCG treatment, which induces ovulation. After the treatments, a remarkable increase of NTS mRNA expression level was observed, increasing the possibility that NTS has a specific effect on spermatozoa. Moreover, using an in vitro cumulus cell culture system, NTS secretion levels were compared to determine which ovarian hormone is responsible for NTS expression. FSH and EGF are known as ovulation inducers. E2 and P4 are typical ovarian hormones. As a result, NTS responds to FSH and EGF, but not to E2 and P4, which increase the secretion levels. Furthermore, a specific inhibitor of MEK, U0126 was used to demonstrate NTS expression is regulated downstream of MAPK in the presence of FSH and EGF. Dose-dependent inhibition of NTS expression was observed in both cases, proving that this pathway works.

In contrast, NTS receptor type 1 is expressed in the neck region of spermatozoa. It is known as a calcium storage, named the redundant nuclear envelope. This localization pattern in NTR1 suggests that NTS induces intracellular calcium mobilization. Because calcium influx into spermatozoa cells is critically important for fertilization, loss of acrosome prior to penetration into the egg membrane is necessary for successful fertilization. Moreover, acrosome reaction is known to be triggered by elevated calcium levels. NTS significantly increased the percentage of acrosome-reacted spermatozoa. Similarly, a recent study discussed the facilitation of acrosome reaction using NTS in the bull and monkey model (Umezu *et al*., 2016; Campbell *et al*., 2020). However, spermatozoa-protein tyrosine phosphorylation is often used as a marker indicating capacitation, defined as spermatozoa which have the ability to fertilize. NTS gradually enhanced tyrosine phosphorylation.

Effects of NTS in preimplantation embryo

Recently, we also revealed the effect of NTS on early embryonic development (Hiradate *et al*., 2020). When the mRNA expression of NTS receptors, *Ntr*1, 2 and 3 was analyzed by qPCR in the preimplantation embryo at each developmental stage, it was found that Ntr1 and 3 were expressed through the blastocyst, suggested NTS can also act on preimplantation embryos. To examine whether NTS affects the development of a pre-implanted embryo, fertilized embryos were cultured in various concentrations of NTS *in vitro*. The ratios of 2-cell and 4-cell embryos were similar, but the Blastocyst formation rate was significantly higher, by as much as 100 nM. The quality of an embryo is also evaluated by counting the number of cells. Comparing this number between the two groups at no supplemented NTS and 100nM NTS added, we found no significant change. Further, the cells of the blastocyst stage can be divided into two types, inner cell mass and trophectoderm. Because ICM cells eventually become the future fetus, The smaller number of ICM makes it difficult for the fetus to develop. Thus, the ratio of the ICM to TE cell number is important. The NTS treatment group showed a higher average, but there was no significant difference. Therefore, these results indicate that the major role of NTS is not proliferation, but rather, differentiation.

Conclusion

We demonstrated that NTS is a novel factor assisting fertilization and early development. This contribution throughout fertilization and early development seems to be a conserved mechanism between species, and it helps to understand the fertility of livestock spermatozoa.

Acknowledgements

I would like to thank the Organizing Committee of the International Virtual Symposium “New Insights on Animal Science”, organized by the International Education and Research Center for Food and Agricultural Immunology (CFAI), and integrated Field Science Center, Graduated School of Agricultural Science, Tohoku University, and JSPS Core-to-Core program.

References

Campbell, G. E., E. L. Jones, P. Comizzoli and D. M. Duffy (2020) Neurotensin stimulates the sperm acrosome reaction and reduces percentages of fertilization in vitro. F & S Science, 1: 27-35.

Carraway, R. E. and S. E. Leeman (1973) The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalami Inflammatory Cytokines View project. The Journal of Biological Chemistry, 248: 6854-6861.

Carraway, R., S. E. Leeman and E. A. Zimmerman (1977) Immunohistochemical Localization of Neurotensin in Endocrine Cells of the Gut. Cell and Tissue Research, 178: 313-321.

Hasan, M. M., J. Vill, F. Lattekivi, J. Ord, Q. U, Ain Reshi, K. Jaager, A. Velthut-Meikas, A. Andronowska, U. Jaakma, A. Salumets and A, Fazeli (2020) Bovine Follicular Fluid and Extracellular Vesicles Derived from Follicular Fluid Alter the Bovine Oviductal Epithelial Cells Transcriptome. International journal of Molecular Science, 21: 53565-53580.

Hiradate, Y., H. Inoue, N. Kobayashi, Y. Shirakata, Y. Suzuki, A. Gotoh, S. G. Roh, T. Uchida, K. Katoh, M. Yoshida, E. Sato and K. Tanemura (2014) Neurotensin Enhances Sperm Capacitation and Acrosome Reaction in Mice. Biology of Reproduction, 91: 1-9.

Hiradate, Y., K. Hara and K. Tanemura (2020) Effect of neurotensin on cultured mouse preimplantation embryos.Journal of Reproduction and Development, 66: 421-425.

Kitabgi, P. (2006) Differential processing of pro-neurotensin/neuromedin N and relationship to pro-hormone convertases. Peptides, 27: 2508-2514

Li, J., J. Song, Y. Y. Zaytseva, Y. Liu, P. Rychahou, K. Jiang, M. E. Starr, J. T. Kim, J. W. Harris, F. B. Yiannikouris, W. S. Katz, P. M. Nilsson, M. Orhomelander, J. Chen, H. Zhu, T. Fahrenholz, R. M. Higashi, T. Gao, L. A. Cassis, T. W. Fan, H. L. Weiss, P. R. Dobner, O. Melander, J. Jia and B. M. Evers (2016) An obligatory role for neurotensin in high-fat-diet-induced obesity. Nature, 533: 411-415.

Reinecke, M. (1987) Neurotensin in the human fallopian tube: Immunohistochemical localization and effects of synthetic neurotensin on motor activity in vitro. Neuroscience Letters, 73: 220-224.

Umezu, K., Y. Hiradate, T. Oikawa, H. Ishiguro, T. Numabe, K. Hara and K. Tanemura (2016) Exogenous neurotensin modulates sperm function in Japanese Black Cattle. Journal of Reproduction and Development, 62: 409-14.