Animal Reproduction Science 149 (2014) 117–123

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

The interactions between nerve growth factor and gonadotrophins in bovine oviduct

Chunjin Li[a,1], Yonghe Ma[a,1], Kangle Yi[c], Chunqiang Wang[b], Wanhong Li[a], Zhuo Liu[a], Lina Sun[a], Shuxiong Chen[a], Jiaxin Yu[a], Hongjiao Li[a], Lu Chen[a,*], Xu Zhou[a,*]

[a] College of Animal Sciences, and Jilin Provincial Key Laboratory of Animal Embryo Engineering, Jilin University, 5333 Xi'an Avenue, Changchun, Jilin Province 130062, PR China
[b] Liaoning Medical University, Jinzhou, Liaoning Province 121001, PR China
[c] Hunan Institute of Animal and Veterinary Science, 8 Changliang Road, Changsha, Hunan Province 410131, PR China

ARTICLE INFO

Article history:
Received 29 March 2014
Received in revised form 30 May 2014
Accepted 23 June 2014
Available online 2 July 2014

Keywords:
Nerve growth factor
Gonadotrophins
Oviduct
Bovine

ABSTRACT

Nerve growth factor promotes the survival and differentiation of nervous cells and is thought to play an important role in the development of reproductive tissues. The aims of this work were to detect the presence of NGF and its receptor NTRK1 in bovine oviduct samples, and to investigate the regulatory interactions between NGF/NTRK1 and gonadotrophins in bovine oviduct epithelial cells. Both transcripts and proteins of NGF and NTRK1 were detected by RT-PCR and Western blotting, and the corresponding proteins were specifically immunolocalized in oviduct epithelial cells. In addition, real-time PCR experiments revealed that the levels of NGF and NTRK1 mRNA in oviduct epithelial cells treated with exogenous FSH or LH were greater than those in negative control cells ($P < 0.05$). Similarly, treatment with NGF significantly increased the expression of FSHR and LHR in oviduct epithelial cells *via* its effects on NTRK1 ($P < 0.05$). This process was suppressed by treatment with the NTRK1 inhibitor K252α. We conclude that NGF/NTRK1 may have a role in regulating the function of bovine oviducts *via* its interactions with gonadotrophins.

1. Introduction

The oviduct is an essential component of the reproductive system in female vertebrates. It has a simple columnar epithelium consisting of ciliated cells and secretory cells (Coy et al., 2012). During follicular development and estrus, the oviduct undergoes specific changes in morphology and endocrinology that create an optimized microenvironment for sperm capacitation, fertilization, embryonic development and embryo transport to the uterus (Buhi et al., 2000). The oviduct fluid is a complex mixture containing materials secreted by the epithelial cells and derived from the blood plasma, including glucose, amino acids, lactate, pyruvate and many growth factors. The concentrations of these substances often change from one physiological stage to another (Buhi et al., 2000).

Changes in the oviduct's secretory behavior seem to be hormonally regulated by ovarian steroids – most importantly, estrogen (Okada et al., 2003). Recent studies using both PCR and immunohistochemical analyses have demonstrated that LHR and FSHR are present in the oviduct, suggesting that gonadotrophins may also contribute to the regulation of oviduct development and secretion (Zheng

* Corresponding authors.
E-mail address: xzhou65@vip.sina.com (X. Zhou).
[1] The authors are co-first authors.

http://dx.doi.org/10.1016/j.anireprosci.2014.06.030
0378-4320/

et al., 1996). Analyses of *in vitro* tissue cultures have shown that luteinizing hormone stimulates the production and release of PGE (2), PGF (2alpha) and ET-1 in the oviducts of cows in the postovulatory phases (Wijayagunawardane et al., 1999 and Wijayagunawardane et al., 2001). Nerve growth factor (NGF) was the first neurotrophin to be identified and plays an essential role in the development of motor and sensory neurons (Thoenen et al., 1987). There is growing evidence to suggest that in addition to its effects on the nervous system, NGF also plays a role in regulating the development of reproductive systems (Abir et al., 2005; Li and Zhou, 2013). The expression of NGF and its receptor neurotrophic tyrosine kinase receptor 1 (NTRK1) in human fetal and adult ovaries (Abir et al., 2005) implies a role for NGF in this organ, while the ability of NGF to stimulate E_2 secretion by human preovulatory ovarian follicles, both directly and by increasing the expression of FSHR (Salas et al., 2006), provides a more precise clue regarding its potential mechanism of action. Interestingly, a recent study showed that both NGF and its receptor were present in the oviducts of golden hamsters (Weng et al., 2009). We have previously reported data suggesting that neurotrophin 4 (NT4), another member of the neurotrophins, was expressed in bovine oviducts and regulated oviduct development by interacting with gonadotrophins (Sun et al., 2012).

Based on these findings, we hypothesized that the NGF might regulate oviduct development by promoting secretory activity among oviduct cells. We have therefore conducted the studies presented herein to investigate the distribution of NGF and its receptor NTRK1 in the bovine oviduct, and explore the interactions between NGF/NTRK1 and gonadotrophins in bovine oviduct epithelial cells using an *in vitro* culture model. The results will provide new evidence of neurotrophins in female reproduction.

2. Methods and materials

2.1. Preparation of bovine oviduct and cell culture

Ten primiparous adult native yellow cows (5 years old Yianbian yellow cow) were studied. Five were in the follicular phase and the others were in the luteal phase. The follicular phase was defined as the period during which the ovary did not have a corpus luteum and the largest follicle was >5 mm in diameter. The luteal phase was defined as the period when the ovary had a corpus luteum (Sun et al., 2012). The animals were slaughtered and their oviducts were separated, placed in a 0.9% solution of NaCl in water containing antibiotics, and transported to the laboratory within 2 h (Sun et al., 2012).

The ampulla part of oviduct in each cow was divided into three parts horizontally with operation scissor. One part was placed in RNA stabilization fluid for RNA extraction and the second was fixed in 4% paraformaldehyde and embedded in paraffin wax for immunohistochemical analysis. In the case of the samples from the follicular phase animals, the third section was used for the isolation of oviduct epithelial cells. In order to minimize the differences between individual tissue samples, the oviduct from each cow was pooled prior to enzymatic digestion.

Table 1
List of primers and probes used for PCR.

Primer and probe	Primer sequence (5′–3′)	Length (bp)
NGF	TCAACAGGACTCACAGGAGCAA	151
	ACCTCTCCCAGCACCATCAC	
TAMRA-FAM	CATCCCACCCCGTCTTTCACCG	
NTRK1	CTGGGTGAGGGTGCCTTT	112
	CGCTCTCAGACACCTCCTTCA	
TAMRA-FAM	TGCCGAGTGCCACAACCTGCTG	
GAPDH	GGCGCCAAGAGGGTCAT	120
	GGTGGTGCAGGAGGCATT	
TAMRA-FAM	TACTTCTCGTGGTTCACGCCCATCACA	

Cells were isolated and cultured using a modified variant of a previously reported procedure (Tiemann and Hansen, 1995). Briefly, after dissection and washing of the external excess tissue with PBS, bovine oviducts were digested in a trypsin-containing medium and then treated with 10% FCS (Fetal Calf Serum) to inhibit further digestion. The digested mixture was centrifuged at $1000 \times g$ for 5 min to collect epithelial cells, whose viability was determined by trypan blue staining prior to culturing. The collected epithelial cells were cultured in 24-well plates at a density of 2×10^4 containing Dulbecco's modified Eagle medium at 38.5 °C under 5% CO_2 for 24 h prior to use in subsequent cell treatments. Each concentration treatment for cells was repeated in duplicate, and the experiment was independently repeated three times.

2.2. Extraction of RNA and RT-PCR

Total RNA from the oviducts and epithelial cells was extracted using Trizol reagent (Invitrogen, Co, Foster, CA, USA). The reverse transcription PCR was performed using a commercial kit (Promega Co., WI, USA) and the resulting cDNA was stored frozen at −20 °C until needed. The specific primers used to amplify cDNA corresponding to the genes encoding NGF and NTRK1 are listed in Table 1. The PCR annealing temperature was 59 °C. The PCR products were directly sequenced using an ABI 377 DNA (Applied Biosystems, Foster City, CA, USA) instrument with M-13F/R primers (TransGenBiotech Co., Beijing, China). The fragments of NGF and NTRK1 were detected in the oviduct of each cow, and the PCR was repeated three times in each cow.

2.3. Western blotting for detecting the NGF and NTRK1 proteins

Western blotting was performed as described previously to detect the proteins of f NGF and NTRK1, and the Western blotting was repeated three times in each cow (Sun et al., 2012). Briefly, total protein was extracted from isolated bovine oviducts using a commercial kit according to the manufacturer's instructions (Applygen Co., Beijing, China). Approximately 50 μg of protein was then separated by SDS-PAGE on a 12% polyacrylamide gel and the separated proteins were transferred onto PVDF membranes (Millipore Co., MA, USA) using a suitable transfer system at 80 V for 2 h. The membranes were blocked by