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—Original—

N-acetylglucosaminyltransferase I Activity of Bovine Oviduct Epithelial Cells: Stimulation by Luteinizing Hormone, Vascular Endothelial Growth Factor and Tumor Necrosis Factor α

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Abstract. *N*-acetylglucosaminyltransferase I (GnT I; EC 2.4.1.101), which catalyzes the first step in the conversion of oligomannose to complex or hybrid *N*-glycans of glycoproteins, was found in media cultured with bovine oviduct epithelial cells (BOEC) obtained from non-pregnant cows during the follicular phase. Combined treatment with specific hormones increased GnT I release from BOEC. Luteinizing hormone (LH; 10 ng/ml) alone slightly, but together with 17β-estradiol (E_2; 1 ng/ml), synergistically increased GnT I activity. Vascular endothelial growth factor (VEGF) and tumor necrosis factor (TNF) α, which have been shown to have their highest activities in the bovine oviduct during the periovulatory period, also increased in GnT I activity. This study provides the first evidence of an increase of GnT I release from BOEC *in vitro*, and shows that endocrine as well as local factors such as LH, VEGF and TNFα increase this activity. The results suggest that GnT I activity in the bovine oviduct may contribute to the induction of glycosylation and thereby contributing to the provision of the optimal microenvironment for fertilization and early development of the embryos.
Key words: Bovine, Luteinizing hormone (LH), *N*-acetylglucosaminyltransferase I, Oviduct, Tumor necrosis factor (TNF) α, Vascular endothelial growth factor (VEGF)

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Many glycoproteins of living organisms contain *N*-linked oligosaccharides (*N*-glycans). Recent advances in cell biology have revealed that many of these *N*-glycans play significant biological roles such as providing signals for cell-surface recognition in multi-cellular organisms [1–3]. Especially, the complicated heterogeneity among three subgroups of *N*-glycans indicates the complex type may be involved in various biological phenomena in mammals. It is known that glycosyltransferases modify the carbohydrate moieties of glycoconjugates by the addition of sugar residues. The conversion of oligomannose *N*-glycans to complex or hybrid *N*-glycans of glycoproteins with antennae structures is catalyzed

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by *N*-acetylglucosaminyltransferase I (GnT I) to GnT VI, as well as α-1,6-fucosyltransferase and β-mannosidases. The GnT I activity catalyzes the initial step in this conversion process [1].

Many glycosyltransferases, in their soluble forms, have been detected in testicular and epididymal fluid [4], ovarian follicular fluid [5] and uterine and oviductal fluid [6], and it has been suggested that they are involved in mammalian fertilization [7]. The level of *N*-acetylglucosaminyltransferase activity is the highest in hamster oviductal fluid during the periovulatory period of the estrous cycle [6]. Moreover, several studies have revealed that the preovulatory surge of luteinizing hormone (LH) activates maximum bovine oviductal secretion and contraction [8–12]. Thus, LH may directly stimulate the release of soluble oviductal glycosyltransferases, increasing glycosylation and thereby providing the best environment for fertilization, gamete/embryo survival and development. However, the capacity of oviduct epithelial cells to secrete/release GnT I and/or activate it, and their possible regulation by endocrine and local factors, are still unknown.

In the present study, the direct effects of LH and 17β-estradiol (E_2) on the activity of soluble GnT I in cultured bovine oviduct epithelial cells (BOEC) were evaluated. We also investigated the possible modulation of bovine oviductal GnT I activity by vascular endothelial growth factor (VEGF) and tumor necrosis factor (TNF) α, which are produced at their highest levels during the periovulatory period and regulate oviduct functions [13–15].

Materials and Methods

Isolation and culture of BOEC

Oviducts removed from non-pregnant Holstein cows during the follicular phase were obtained from a local slaughterhouse. The stage of the estrous cycle was identified as described in previous reports [16, 17]. The oviducts were transported to the laboratory in ice-cold Hank's balanced salt solution (HBSS; Nissui pharmaceutical Co., Ltd., Tokyo, Japan) and BOEC were immediately isolated in accordance with the following process [9, 10].

Briefly, the surrounding connective tissues were trimmed from the oviduct, the oviductal lumen was washed with 10 ml HBSS and BOEC were mechanically dislodged while flushing with 15 ml HBSS. The collected BOEC were suspended in HBSS and centrifuged at 300 × g for 10 min at 4 C. The cell pellet was suspended in 4 ml HBSS, layered over 5 ml Percol (Sigma and Aldrich Chemical Co., St. Louis, MO, USA) and centrifuged at 900 × g for 20 min at 4 C. The cells in interphase were collected and washed twice with HBSS. The collected BOEC were cultured overnight in M 199 with 5% (v/v) fetal bovine serum (FBS; Bio Whittaker, Walkersville, MD, USA) at 38.5 C in 5% CO_2 and 95% humidity. The BOEC still in suspension were taken, washed twice with HBSS and trypsinized [0.05% (w/v) trypsin; Amresco, Salon, OH, USA] until single cells appeared. The BOEC were plated in 6-well culture dishes (Nalge Nunc International, Roskilde, Denmark) and incubated under standard culture conditions. After monolayer formation, the cells were again trypsinized and plated in 6-well culture dishes at a density of 3×10^4/ml, and cultured until sub-confluent.

Hormone treatments

The sub-confluent BOEC were washed twice with M 199 supplemented with 1% (w/v) BSA (Sigma and Aldrich), and incubated for 24 h with bovine LH (10 ng/ml; USDA-bLH-B-6, USDA Animal Hormone Program, USA), E_2 (1 ng/ml; Sigma and Aldrich), bovine LH+E_2 or 3 different concentrations of human VEGF (Pepro Teches Ltd., London, UK; 0.1, 1 and 10 ng/ml), or human TNFα (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan; 0 .1, 1 and 10 ng/ml). All 6 wells of a single culture dish were used for each experiment and each experiment was repeated 5 times using different sources of BOEC.

The concentrations of E_2 used in this study were based on those detected in the cow oviduct during the normal estrous cycle [16, 17]. The average time taken for BOEC to form a monolayer was 5 days. The cell viability was estimated using Trypan-blue staining; it was found to be greater than 90% at each time of plating as well as at the end of each experiment. The average cell concentration at the end of each experiment was 8×10^4 cells/ml.

Determination of GnT I activity

After incubation for 24 h, the medium was collected and its GnT I activity was assayed by

measuring the incorporation of [^{3}H]-GlcNAc into the acceptor substrate, α1-3α1-6 mannopentaose, as described previously [5]. The assay mixture (100 μl) consisted of 50 mM sodium cacodylate buffer (pH 5.8), 50 mM $CoCl_2$, 2 mM ATP (Sigma and Aldrich), 200 mM D-*N*-acetylglucosamine (D-GlcNAc, Sigma and Aldrich), 1.5 mM UDP-GlcNAc (Sigma and Aldrich) containing UDP-[^{3}H]-GlcNAc (PerkinElmer, Inc., MA, USA; 100 kBq/ml incubation mixture), 6.7 mM α1-3α1-6 mannopentaose ([Man(α1-6)[Man(α1-3)]Man(α1-6)[Man(α1-3)Man]; Funakoshi Co., Tokyo, Japan] and 10 μl of culture medium. Parallel incubations without acceptor were performed as controls. After incubation for 4 h at 38.5 C, the mixture was cooled in ice to stop the enzyme reaction and immediately passed through 4 ml of AG1-X8 ion exchange resin (Bio-Rad Laboratories, Hercules, CA, USA; Cl^- form, 200–400 mesh), contained in a Pasteur pipette, to remove residual UDP-[^{3}H]-GlcNAc. Immediately after the elution, the incorporation of [^{3}H]-GlcNAc into the acceptor was measured by liquid scintillation counting using a Tri-Carbo scintillation counter (PerkinElmer). The concentration of protein in the media supernatant was determined by the Lowry method [18] using bovine serum albumin as the standard.

Statistical analysis

For data analysis, the amount of radioactivity detected in the control group, which was co-cultured with neither hormones nor cytokines, was used to calculate the baseline (defined as 100%) and the amount of radioactivity detected in each treatment was expressed as a percentage of the corresponding baseline. The effect of the different treatments on the GnT I activity in the culture medium were compared with control groups using Student's *t*-test. Probabilities less than 5% ($P<0.05$) were considered statistically significant.

Results

The GnT I activity detected in the media supernatant cultured with BOEC in the absence of hormones and cytokines was 0.11 ± 0.012 nM products/h/mg protein. This activity can be compared with that of bovine follicular fluid (12.2 ± 2.0 nM products/h/mg protein) [5] and of rat testicular and epididymal fluids (0.23 ± 0.02 and 0.26 ± 0.02 nM products/h/mg protein, respectively) [4].

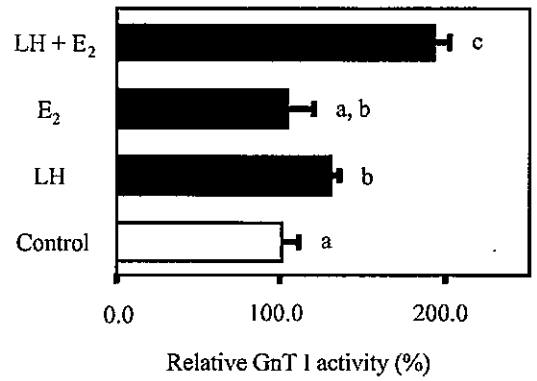

Fig. 1. Effect of LH (10 ng/ml), E_2 (1 ng/ml) and LH+E_2 on the relative GnT I activity (n=4, mean ± SEM) in the media cultured with bovine oviduct epithelial cell (BOEC) monolayers for 24 h. Different letters above the bars denote a significant difference (a vs. b: $P<0.05$; a vs. c : $P<0.01$; and b vs. c: $P<0.05$).

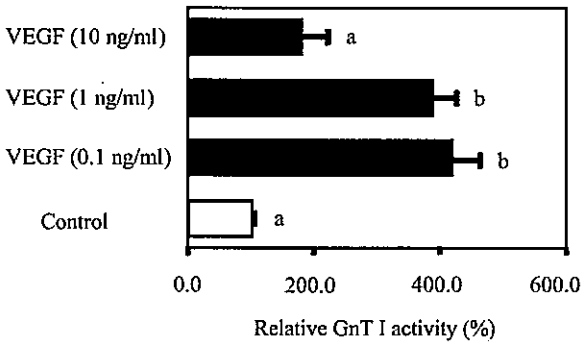

Fig. 2. Effect of various doses of VEGF on the relative GnT I activity (n=5, mean ± SEM) in the media cultured with BOEC monolayers for 24 h. Different letters above the bars denote a significant difference ($P<0.01$).

Effects of LH and E_2 on the GnT I release from BOEC

LH increased GnT I release from BOEC after 24 h culture ($P<0.05$), but E_2 had no effect (Fig. 1). The combined treatment of LH+E_2 synergistically increased GnT I activity ($P<0.01$).

Effects of VEGF and TNF α on the GnT I release from BOEC

Addition of 0.1 ng/ml VEGF resulted in a more than 4-fold increase in GnT I activity ($P<0.01$). This

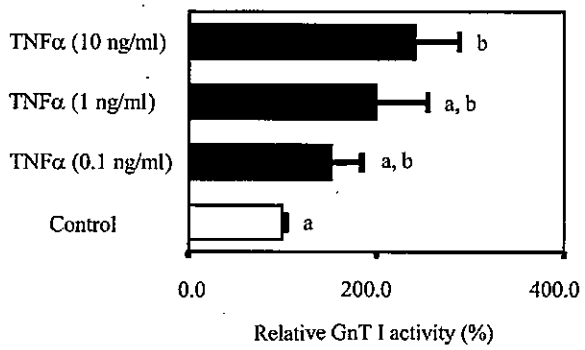

Fig. 3. Dose-dependent response of TNFα on the relative GnT I activity (n=4, mean ± SEM) in the media cultured with BOEC monolayers for 24 h. Different letters above the bars denote a significant difference ($P<0.01$).

stimulation was very similar to that with 1 ng/ml VEGF after 24 h culture (Fig. 2). However, the highest concentration of VEGF (10 ng/ml) had no effect compared with the control group.

TNFα does-dependently increased GnT I activity after 24 h culture ($P<0.05$; Fig. 3). The highest concentration of TNFα (10 ng/ml) produced a two-fold increase compared with the control value.

Discussion

The results of the present study provide evidence that soluble GnT I is expressed by BOEC in culture media, and that LH together with E_2 strongly increases GnT I release from BOEC *in vitro*. Moreover, the local cytokines, VEGF and TNFα, also increased GnT I activity, suggesting that they regulate the glycosylation occurring in the microenvironment of the oviduct, particularly during the periovulatory period of the estrous cycle.

Glycosyltransferases are generally classified as type II membrane protein and are localized in the Golgi apparatus [19]. Some of these enzymes are released through cleavage by endogenous proteases, and then secreted from the cell [20]. Indeed, the fact that glycosyltransferase activities are detected in various body fluids has been well documented [19]. In the hamster oviduct, a temporal surge of glycosyltransferase activity was observed at the onset of ovulation, suggesting that glycoprotein-modifying enzymes regulate the carbohydrate moieties of glycoproteins on the sperm plasma membrane as well as on zona pellucida glycoproteins at the site of fertilization. Alternatively they may alter the surface glycoproteins of the fertilized egg in the uterus prior to implantation [7]. In support of such a high level of glycosyltransferase activity observed in the hamster oviduct, the present study showed that a combination treatment of LH and E_2 significantly increased GnT I release from BOEC. However, it is still not known whether glycoprotein-modifying enzymes act on the epithelial cell surfaces or on the soluble peptides present in the oviductal fluid, since the presence of sugar nucleotides in oviductal fluid has not yet been demonstrated. Further studies are required to elucidate this point.

In the female reproductive system, the appropriate biological activity of VEGF and TNFα are crucial for the cascade of events that produce a successful pregnancy [8, 13, 15, 21–23]. The local content of VEGF in the bovine oviduct is at its highest during the periovulatory period [8], and may be responsible for the higher fluid secretion within the oviduct during this period [24]. Moreover, we recently reported that VEGF may contribute to regulation of the different contractile patterns of the oviduct, which occur during the period of gamete and embryo transport [15]. TNFα and its receptors are highly expressed in the cow oviduct during the periovulatory period, and may act to optimize the release of contraction-related substances during this period [13]. These observations indicate that local VEGF and TNFα systems are activated during the periovulatory period. Furthermore, we demonstrated in the present study that both VEGF and TNFα directly increased GnT I release from BOEC. Thus, we consider that VEGF and TNFα act synergistically to increase the release of GnT I in the oviduct which is triggered by LH and E_2 during the periovulatory period *in vivo*.

In conclusion, we provide the first evidence that bovine oviduct epithelial cells release activated GnT I *in vitro*, and that endocrine as well as local factors such as LH, VEGF and TNFα stimulate this release. We suggest that the GnT I activity in the oviduct may contribute to the induction of active glycosylation which is related to the provision of the optimal microenvironment for fertilization and early development of embryos.

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References

1. **Kobata A.** Structures and functions of the sugar chains of glycoproteins. *Eur J Biochem* 1992; 209: 483–501.
2. **Rademacher TW, Parekh RB, Dwek RA.** Glycobiology. *Annu Rev Biochem* 1988; 57: 785–838.
3. **Schachter H.** Congenital disorders involving defective *N*-glycosylation of proteins. *Cell Mol Life Sci* 2001; 58: 1085–1104.
4. **Nozaki H, Matsuzawa T, Nakamura T, Arai I, Urashima T.** Are there two forms of beta 2-*N*-acetylglucosaminyltransferase I in rat testicular and epididymal fluids? *Biochim Biophys Acta* 2003; 1649: 140–145.
5. **Nozaki H, Miyamoto A, Hayashi KG, Matsui M, Yoshida T, Matsuzawa T, Nakamura T, Arai I, Urashima T.** *N*-acetylglucosaminyltransferase I activity in bovine ovarian follicular fluids from dominant and atretic follicles. *J Appl Glycosci* 2004; 51: 315–320.
6. **Tulsiani DR, Chayko CA, Orgebin-Crist MC, Araki Y.** Temporal surge of glycosyltransferase activities in the genital tract of the hamster during the estrous cycle. *Biol Reprod* 1996; 54: 1032–1037.
7. **Tulsiani DR, Yoshida-Komiya H, Araki Y.** Mammalian fertilization: a carbohydrate-mediated event. *Biol Reprod* 1997; 57: 487–494.
8. **Gabler C, Einspanier A, Schams D, Einspanier R.** Expression of vascular endothelial growth factor (VEGF) and its corresponding receptors (flt-1 and flk-1) in the bovine oviduct. *Mol Reprod Dev* 1999; 53: 376–383.
9. **Wijayagunawardane MP, Miyamoto A, Sato K.** Prostaglandin E_2, prostaglandin F_2 alpha and endothelin-1 production by cow oviductal epithelial cell monolayers: effect of progesterone, estradiol 17 beta, oxytocin and luteinizing hormone. *Theriogenology* 1999; 52: 791–801.
10. **Wijayagunawardane MP, Choi YH, Miyamoto A, Kamishita H, Fujimoto S, Takagi M, Sato K.** Effect of ovarian steroids and oxytocin on the production of prostaglandin E_2, prostaglandin F_2alpha and endothelin-1 from cow oviductal epithelial cell monolayers *in vitro*. *Anim Reprod Sci* 1999; 56: 11–17.
11. **Wijayagunawardane MP, Miyamoto A, Taquahashi Y, Gabler C, Acosta TJ, Nishimura M, Killian G, Sato K.** *In vitro* regulation of local secretion and contraction of the bovine oviduct: stimulation by luteinizing hormone, endothelin-1 and prostaglandins, and inhibition by oxytocin. *J Endocrinol* 2001; 168: 117–130.
12. **Wijayagunawardane MP, Miyamoto A, Taquahashi Y, Acosta TJ, Nishimura M, Sato K.** Angiotensin II and atrial natriuretic peptide in the cow oviductal contraction *in vitro*: direct effect and local secretion of prostaglandins, endothelin-1, and angiotensin II. *Biol Reprod* 2001; 65: 799–804.
13. **Wijayagunawardane MP, Gabler C, Killian G, Miyamoto A.** Tumor necrosis factor alpha in the bovine oviduct during the estrous cycle: messenger RNA expression and effect on secretion of prostaglandins, endothelin-1, and angiotensin II. *Biol Reprod* 2003; 69: 1341–1346.
14. **Wijayagunawardane MP, Miyamoto A.** Tumor necrosis factor alpha system in the bovine oviduct: a possible mechanism for embryo transport. *J Reprod Dev* 2004; 50: 57–62.
15. **Wijayagunawardane MP, Kodithuwakku SP, Yamamoto D, Miyamoto A.** Vascular endothelial growth factor system in the cow oviduct: a possible involvement in the regulation of oviductal contraction and embryo transport. *Mol Reprod Dev* 2005: (In press).
16. **Wijayagunawardane MPB, Cerbito WA, Miyamoto A, Acosta TJ, Takagi M, Miyazawa K, Sato K.** Oviductal progesterone concentration and its spatial distribution in cyclic and early pregnant cows. *Theriogenology* 1996; 46: 1149–1158.
17. **Wijayagunawardane MP, Miyamoto A, Cerbito WA, Acosta TJ, Takagi M, Sato K.** Local distributions of oviductal estradiol, progesterone, prostaglandins, oxytocin and endothelin-1 in the cyclic cow. *Theriogenology* 1998; 49: 607–618.
18. **Lowry OH, Rosebrough NJ, Farr AL, Randall RJ.** Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193: 265–275.
19. **Paulson JC, Colley KJ.** Glycosyltransferases. Structure, localization, and control of cell type-specific glycosylation. *J Biol Chem* 1989; 264: 17615–17618.
20. **Kitazume S, Tachida Y, Oka R, Shirotani K, Saido**

TC, Hashimoto Y. Alzheimer's beta-secretase, beta-site amyloid precursor protein-cleaving enzyme, is responsible for cleavage secretion of a Golgi-resident sialyltransferase. *Proc Natl Acad Sci USA* 2001; 98: 13554–13559.

21. **Gordon J, Mesiano S, Zaloudek C, Jaffe R.** Vascular endothelial growth factor localization in human ovary and fallopian tubes: possible role in reproductive function and ovarian cyst formation. *J Clin Endocrinol Metab* 1996 Jan; 81: 353–359.
22. **Berisha B, Schams D, Kosmann M, Amselgruber W, Einspanier R.** Expression and localisation of vascular endothelial growth factor and basic fibroblast growth factor during the final growth of bovine ovarian follicles. *J Endocrinol* 2000; 167: 371–382.
23. **Cheung CY.** Vascular endothelial growth factor: possible role in fetal development and placental function. *J Soc Gynecol Investig* 1997; 4: 169–177.
24. **Kavanaugh JF, Killian GJ.** Bovine oviductal cannulations. *J Invest Surg* 1988; 1: 201–208.