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Gastrin-Releasing Peptide Receptor (GRPR) in the Bovine Uterus and Placenta

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Abstract: Gastrin-releasing peptide (GRP), which is a 27 amino acid peptide, the mammalian homologue of bombesin, has been identified in various organs including the uterus and placenta. The effects of GRP are mediated by the gastrin-releasing peptide receptor (GRPR), one of the seven transmembrane-spanning G protein-coupled receptors. Although the localization of GRP has been reported in each cell type of the placentae that of GRPR currently remains unknown. Therefore, the aim of the present study was to immunohistochemically examine the localization of GRPR in the bovine uterus and placenta. In the placental tissues, GRPR immunoreactivity was detected in the cytoplasm of uninucleate trophoblast cells (trophoblast cells), but not in binucleate trophoblast giant cells or maternal tissues including the uterine glands. In nonpregnant animals, GRPR was localized in the endometrial epithelial cells of the caruncle only. The differences observed in the localization of GRPR in the chorionic epithelium in the present study demonstrated that GRP directly affected trophoblast cells, but not binucleate trophoblast giant cells differentiated from trophoblast cells. In the present study, the localization of GRPR in the bovine placenta demonstrated is the first in mammalian placentas.
Keywords: GRPR, uterus, placenta, bovine.

INTRODUCTION

Gastrin-releasing peptide (GRP) is the a 27 amino acid peptide, the mammalian homologue of bombesin with a wide range of bioactivities, including regulation of the digestive system, such as the stimulated release of gastrointestinal hormones, modulation of gastrointestinal motility and promotion of pancreatic secretions [1, 2].

The effects of GRP are mediated by a specific seven transmembrane-spanning G protein-coupled receptor, the gastrin-releasing peptide receptor (GRPR) [3-5]. GRP are widely expressed in central and enteric nervous systems as neuropeptides [6] and regulate normal physiological functions, such as satiety [7], thermoregulation [8, 5], circadian rhythms [9], smooth muscle contraction [10, 11], the release of other peptide hormones [1, 12] and endometrial ion transport [13] through GRPRs on the cell membrane of each target cell.

GRP acts as a growth factor in tumor cells [14-17] and also in normal tissues, for example, the gastrointestinal tract [18-21], pancreas [22-24] and other tissues [25, 26]. GRP has also been detected in reproductive organs, and the abundant expression of GRP has been reported in the uterus and/or placenta of some mammals, including humans [27, 28], sheeps [29], cattles [30] and opossums [31]. These findings suggested the autocrine and/or paracrine effects of GRP in uterine and placental tissues. However, GRPR mRNA is not expressed in the ovine pregnant endometrium and cotyledonary placenta, whereas very low expression levels of GRPR have been detected in the conceptus [31]. The aim of the present study was to investigate the GRPR localization in the bovine uterus and placenta. We herein demonstrated that in bovine, GRP and GRPR were localized in the endometrial epithelial cells of nonpregnant caruncle, placental trophoblast cells and provided evidence to support this peptide hormone acting as an autocrine and or paracrine factor in uterine and placental tissues.

MATERIALS AND METHODS

Fourteen placentas and 6 nonpregnant of adult bovine uteri were used in the present study. The gestational day (51- to 251-day of pregnancy) of samples was estimated from the crown-rump length of fetuses (CRL5-90 cm) [32]. Samples were obtained from a local slaughterhouse. Tissue samples were collected from the caruncle and intercaruncle of the uterus, and from the placentome (comprising the caruncle and cotyledon) and interplacentome. After

fixation in Bouin's fluid for 24 hr, tissue samples were dehydrated in ethanol, cleared in xylene, embedded in paraffin (Paraplast 6 Plus®, Kendall, MA, USA) and cut serially at a thickness of 4 μm.

The ImmPRESS™ polymerized reporter enzyme staining system (Vector Laboratories, Inc., Burlingame, CA, USA) was employed for immunohistochemical detection. Tissue sections were deparaffinized in xylene, rehydrated in descending series of ethanol concentrations, washed in distilled water (DW), and then treated in target retrieval solution (1:10, S1699; DakoCytomation, Inc., Carpintaria, CA, USA) for 15 min at 95 ºC to retrieve antigens. After washing in DW, sections were incubated with 0.3% H_2O_2 in methanol for 10 min at room temperature (RT) to block endogenous peroxidase activity. After a treatment with normal horse serum for 30 min at RT, sections were incubated overnight with a rabbit anti-human GRPR antibody (1:500, GTX13339, GeneTex, Inc., San Antonio, USA) at 4 ºC in a moisture chamber. After incubation with a primary antibody, ImmPRESS horse anti-rabbit IgG (ImmPRESS™ reagent, MP-7401, Vector Laboratories, Inc.) was applied as the secondary antibody for 30 min at RT. Binding sites were then visualized by acetonitrile (Vector® SG Substrate Kit, SK-4700, Vector Laboratories, Inc.) or 0.02% 3,3'-diaminobenzidine tetrahydrochloride (DAB) in 50 mM Tris-HCl (pH 7.4) containing 0.006% H_2O_2. The sections visualized by DAB were counterstained with Mayer's hematoxylin. Negative control sections were treated with the omission of the primary antibody. Immunostained sections were examined with a conventional light microscope, and photomicrographs were taken with a digital camera (Digital Sight DS-5M, Nikon, Tokyo, Japan).

RESULTS AND DISCUSSION

GRPR immunoreactivity was detected in bovine uteri and placentas (placentomes). In pregnant bovine, GRPR immunoreactivity was present in the cytoplasm of uninucleate trophoblast cells (trophoblast cells) that lined the chorionic villi of the cotyledon, the so-called fetal placenta (Fig. 1A & B). However, the immunoreactivity was not detected in binucleate trophoblast giant cells (trophoblast giant cells) or the trophoblast cells of the intercotyledon (Fig. 1A & B). Moreover, GRPR immunoreactivity was absent in endometrial tissues including the uterine glands of the caruncular (maternal placenta) and intercaruncular parts (Fig. 1A-C).

In nonpregnant bovine, GRPR was localized in the endometrial epithelial cells of the caruncle (Fig. 1D), but not in those of the intercaruncle. Furthermore, GRPR immunoreactivity was not detected in the uterine glandular cells, similar to pregnant animals. Immunoreactivity for GRPR was absent in negative controls.

GRPR has been identified in human cancer cell lines of the lung [15], breast [16], prostate [17] and colon [33]. Furthermore, the expression of GRPR in various human tumors was summarized by Cornelio *et al*. [34]. In normal human tissues, GRPR has been detected in intestinal smooth muscle cells [35], the colonic mucosal epithelium during gut development [18], breast tissue [36], the kidney [37] and prostate [38]. Moreover, the expression of GRPR was previously reported in the myometrium, uterine glands, and endometrial blood vessels of nonpregnant human uteri [39] and also in the human uteroplacental tissue; however, the cell types expressing GRPR were not identified [27]. GRPR has also been identified in the rat uterus [40, 41] However, few studies have described the localization of GRPR in the female genital organs of domestic animals. We herein clearly demonstrated immunohistochemically, the localization of GRPR in uterine and placental tissues.

In this study on the nonpregnant bovine uterus, GRPR immunoreactivity was localized in the endometrial epithelial cells of caruncle only. In the bovine placenta, GRPR immunoreactivity was detected in trophoblast cells, but not in binucleate trophoblast giant cells. On the other hand, GRPR was absent in the uterine glandular cells of bovine uterine and placental tissues. The absence of GRPR in trophoblast giant cells and glandular epithelial cells was contrary to our expectations because we previously reported the abundant expression of GRP in the binucleate trophoblast giant cells and glandular epithelial cells of nonpregnant and pregnant uteri [30, 42] and the autocrine and paracrine feedback systems among the same cell types were expected. Therefore, the secretion of GRP from trophoblast giant cells and glandular epithelial cells may be regulated by other factors, unlike the autocrine and paracrine systems through GRPR. On the other hand, the localization of GRP [30, 42] and GRPR (in the present study) was demonstrated in the endometrial epithelial cells of nonpregnant caruncle and placental trophoblast cells, suggesting the regulation among the same cell types and/or that by other GRP sources, such as uterine glandular cells and trophoblast giant cells. Previous studies showed that GRP was mainly produced by uterine glandular cells in nonpregnant and pregnant cows [30, 42]. Therefore, GRPR-positive cells such as trophoblast cells and endometrial epithelial cells may predominantly be affected by GRP secreted from uterine glandular cells.

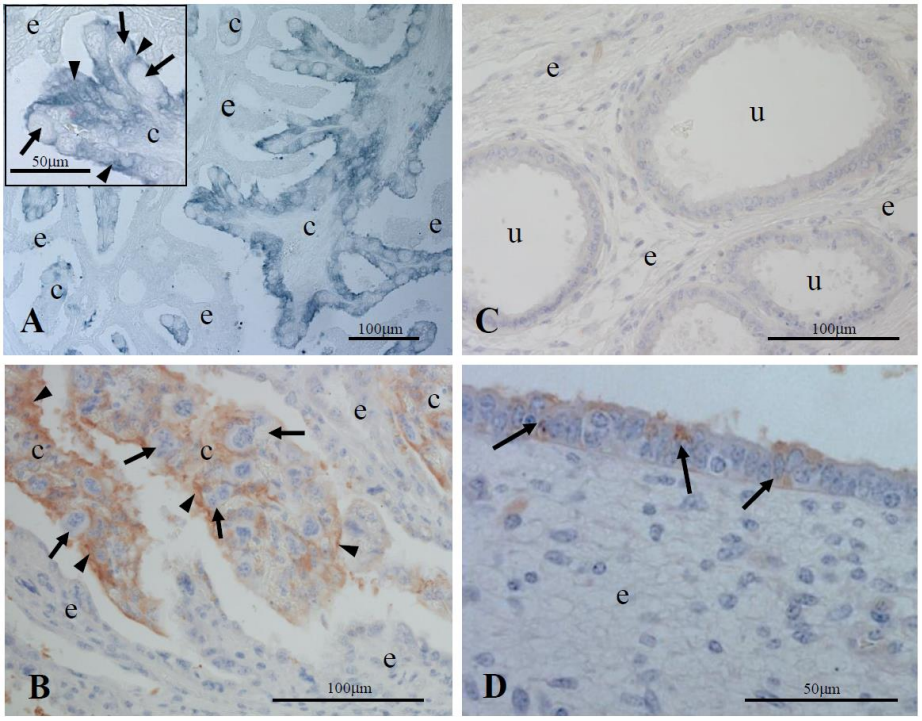

Fig-1: Immonohistochemical localization of GRPR in the bovine placenta and uterus. A: Placenta (194-day/CRL60 cm). The section shows a higher magnification (x520) of A. B: Placenta (251-day/CRL90 cm). C: Endometrial uterine glands below the placentome (251-day/CRL 90 cm). D. Caruncle of nonpregnant uterus. Immunoreactivity for GRPR was detected in the uninucleate trophoblast cells (arrow heads) and endometrial epithelial cells (large arrows), but not in binucleate trophoblast giant cells (small arrows) or uterine glandular cells. c: chorionic villi, e: endometrium, u:uterine gland. Coloring of acetonitrile (A) and DAB (B-D)

Ruminants have a synepitheliochorial placenta in which binucleate trophoblast giant cells are formed in the chorionic epithelium, migrate towards the endometrium and fuse with endometrial epithelial cells, producing fetomaternal hybrid syncytial cells [43]. Binucleate trophoblast giant cells have been shown to solely produce several proteins, such as placental lactogen, prolactin-related protein-1, pregnancy-associated glycoproteins (PAG-1 subgroup) and estrogen [44, 45, 43, 46]. In the present study, the GRPR expression in the placenta was only detected in trophoblast cells, and not in trophoblast giant cells. Therefore, GRP may not be directly involved in the maturation or functions of binucleate trophoblast giant cells. Binucleate trophoblast giant cells are considered to develop from uninucleate trophoblast cells by acytokinetic mitoses [47, 48, 49]. Trophoblast giant cells are formed from uninucleate trophoblast cells by two subsequent mitoses [43, 50]; the first mitosis of a uninucleate trophoblast cell produces two cells, one without apical tight junctions, and this cell continuously undergoes acytokinetic mitosis, which leads to a binucleate cell with diploid nuclei. GRP is known to possess a mitogenic capacity in normal tissues [18, 19, 22, 2]. Thus, we speculated that GRPR on uninucleate trophoblasts cells may be involved in an acytokinetic mitosis process for the formation of binucleate trophoblast giant cells. However, previous studies reported that, in culture with GRP-free medium, bovine trophoblast cell line (BT-1) cells were induced into binucleate trophoblast giant cells that synthesize placental lactogen [51, 52]. Therefore, GRP may merely be involved in the acceleration of uninucleate trophoblast cell proliferation. The disappearance of GRPR in binucleate trophoblast giant cells may further inhibit cell division in order to initiate their maturation and migration.

The endometrium is a complex tissue and mainly consists of epithelial and stromal cells [53]. The physiological function of epithelial and stromal cells currently remains unclear. Progesterone (P4), estrogens, and oxytocin have been shown to mainly regulate morphological and functional changes in the endometrium throughout the estrous cycle [54, 55]. The secretory cells of the endometrial epithelium produce and release mucus on the epithelial surface [54, 56]. A previous study reported that the secretion of mucus in the nasal mucosa was stimulated by GRP [57]. Moreover, the secretion of mucus from the bronchial mucosa has been suggested to be induced by the stimulation of GRP binding to the submucosal glands and epithelium [58]. In the present study on bovine uteri, GRPR was immunolocalized in endometrial epithelial cells, which suggested that the expression of GRPR was involved not only in autocrine and paracrine feedback systems, but also in the production of secretions including mucus. In addition, the

proliferation of endometrial epithelial cells may also be induced by GRP because of its ability to promote cell division.

CONCLUSION

In conclusion, we here demonstrated that GRPR was localized in the uterine epithelial cells of nonpregnant and uninucleate trophoblast cells of pregnant bovine, verifying the autocrine and paracrine actions of GRP in bovine uteri and placentas.

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