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Structure of the bovine *VASAP-60/PRKCSH* gene, functional analysis of the promoter, and gene expression analysis

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Abstract

Vacuolar system-associated protein-60 (VASAP-60) constitutes the bovine ortholog of the human "protein kinase C substrate 80K-H" (PRKCSH or 80K-H). We characterized the bovine *VASAP-60/PRKCSH* gene structure and promoter, identified *cis*-acting elements controlling *VASAP-60* expression, searched for mRNA splice variants, and analyzed mRNA expression in ovarian follicles. Expression of *VASAP-60* mRNA showed a 2.4-fold increase ($P<0.0001$) in granulosa cells of dominant follicles compared to small follicles (2–4 mm) or ovulatory follicles, and no mRNA splice variant was identified. The bovine *VASAP-60* gene encompasses 12.5 kb and is composed of 18 exons and 17 introns. Primer extension analysis revealed a single transcription initiation site, and the promoter lacks a TATA box. Promoter activity assays were performed with a series of deletion constructs in different bovine cell lines (endometrial epithelial glandular, kidney epithelial and aortic endothelial) to identify *cis*-acting elements. The −53/+16 bp fragment (+1 = transcription start site) conferred minimal promoter activity whereas activator and repressor elements were located in the −200/−53 bp and −653/−200 bp fragments, respectively. Analysis of *cis*-acting elements in the −200/−53 bp activation domain revealed by gel shift assays and chromatin immunoprecipitation assay that transcription factor YY1 binds to *VASAP-60* promoter. This study is the first to report that *VASAP-60* is up-regulated in granulosa cells of dominant follicles, to document the primary structure of the bovine *VASAP-60* gene and promoter, and to demonstrate that YY1 binds to the *VASAP-60* proximal promoter and may act as a positive transcriptional regulator.

Keywords: VASAP-60; 80K-H; PRKCSH; Beta-glucosidase II; Hepatocystin; YY1

1. Introduction

Vacuolar system-associated protein-60 (VASAP-60) was first characterized from bovine ovarian granulosa and luteal cells (Brûlé et al., 2000). It was named VASAP-60 based on its apparent localization in the Golgi/endosomal fraction and its association with intracellular vesicles other than the endoplasmic reticulum (ER). Amino acid comparison of bovine VASAP-60 to that of other species indicates that it is the bovine ortholog of the human "protein kinase C substrate 80K-H" (PRKCSH or 80K-H) (Sakai et al., 1989). The PRKCSH/80K-H protein was also named the non-catalytic beta subunit of glucosidase II (βGII) (Trombetta et al., 1996; Arendt and Ostergaard, 1997) and advanced glycation end products-receptor 2 (AGE-R2) (Li et al., 1996; Iacobini et al., 2003). To date, the biological role of VASAP-60/PRKCSH remains unclear. Possible functions include roles in the formation of membrane protein complexes involved in signal transduction of fibroblast growth factor receptor 3 (Goh et al., 1996; Kanai et al., 1997; Forough et al., 2003), intracellular signaling (Hirai and Shimizu, 1990), as AGE-R2 receptor (Li et al., 1996; Iacobini et al., 2003),

Abbreviations: AGE-R2, advanced glycation end products-receptor 2; αGII, alpha subunit of glucosidase II; βGII, beta subunit of glucosidase II; CL, corpora lutea; DF, dominant follicle; ER, endoplasmic reticulum; FGF-1, fibroblast growth factor-1; GAPD, glyceraldehyde-3-phosphate dehydrogenase; GLUT4, glucose transporter 4; hCG, human chorionic gonadotropin; OF, ovulatory follicle; PRKCSH, protein kinase C substrate 80K-H; NBC, newborn calf serum; SF, small follicle; TRPV5, Ca^{2+} sensor and regulator of the epithelial transient receptor potential cation channel subfamily V member 5; USF1, upstream stimulatory factor-1; USF2, upstream stimulatory factor-2; VASAP-60, vacuolar system-associated protein-60; YY1, transcription factor Yin Yang 1.

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intracellular trafficking events (Brûlé et al., 2000, 2003), as a non-catalytic β subunit of αGII (Trombetta et al., 1996; Arendt and Ostergaard, 1997; Flura et al., 1997; Hentges and Bause, 1997; Pelletier et al., 2000), as a Ca^{2+} sensor and regulator of the epithelial transient receptor potential cation channel subfamily V member 5 (TRPV5) (Gkika et al., 2004), and as being involved in glucose transporter 4 (GLUT4) trafficking (Hodgkinson et al., 2005). Autosomal dominant polycystic liver disease in humans has recently been associated with multiple mutations in the open reading frame of the *PRKCSH/80K-H* gene resulting in premature chain termination and causing a loss of function of the PRKCSH/80K-H protein (Li et al., 2003; Drenth et al., 2004; Davila et al., 2004). The name hepatocystin was also suggested for the gene in relation to the phenotype described in man (Everson et al., 2004). Immunocytochemical observations of VASAP-60 showed that the protein mainly localized in the perinuclear area in agreement with an ER localization (Brûlé et al., 2000, 2003; Treml et al., 2000). However, it was shown that VASAP-60 can be partially proteolyzed into a 58 kDa truncated form lacking its carboxyl terminus thought to allow its translocation to other cellular membrane compartments (Brûlé et al., 2003). Immunolocalization of VASAP-60 has also shown a labeling pattern associated with other components of the intracellular vesicular network suggesting that VASAP-60 may be more generally involved in intracellular transport events in addition to being a component of the ER compartment (Brûlé et al., 2003; Gkika et al., 2004). Treatment of human mammary carcinoma epithelial cells with fibroblast growth factor-1 (FGF-1) was shown to stimulate the translocation of PRKCSH/80K-H from the cytoplasm to the nucleus (Forough et al., 2003). VASAP-60/PRKCSH was shown to interact with proteins like αGII (Trombetta et al., 1996; Arendt and Ostergaard, 1997; Flura et al., 1997; Hentges and Bause, 1997; Pelletier et al., 2000; Brûlé et al., 2003), proteins involved in signal transduction (Goh et al., 1996; Kanai et al., 1997), TRPV5 (Gkika et al., 2004), GLUT4 (Hodgkinson et al., 2005), and other unidentified proteins (Brûlé et al., 2003).

To further our understanding of the relation between VASAP-60/PRKCSH expression and its biological function, we undertook the characterization of the bovine gene. Since mRNA isoforms were reported for the mouse and human *80K-H/βGII* mRNAs (Arendt et al., 1999; Treml et al., 2000), we compared gene structure to cDNA structure. *VASAP-60/PRKCSH* mRNA was shown to be expressed in multiple tissues (Brûlé et al., 2000; Li et al., 2003) but no information is available as to whether mRNA expression levels vary for a given tissue or cell type in relation to developmental status or physiological condition. We thus studied the expression of *VASAP-60* mRNA in granulosa cells obtained from ovarian follicles collected at different stages of development since VASAP-60 was first characterized from granulosa and luteal cells (Brûlé et al., 2000). Also, since VASAP-60/PRKCSH is known to interact with αGII, the expression of *αGII* mRNA was analyzed in parallel with *VASAP-60/PRKCSH*. Since nothing is known about the transcriptional control of this gene in any cell type or species, we investigated the function of *VASAP-60/PRKCSH* promoter using bovine endometrial epithelial glandular, kidney epithelial and aortic endothelial cell lines, and provided evidence that the transcription factor Yin Yang 1 (YY1) binds to *VASAP-60/PRKCSH* proximal promoter.

2. Materials and methods

2.1. In vivo experimental model and RNA isolation

Bovine ovarian follicles and corpora lutea (CL) were isolated at specific stages of the estrous cycle from normal cyclic crossbred heifers, as described (Bédard et al., 2003). Briefly, estrous cycle was synchronized with $PGF_{2\alpha}$ (25 mg, im; Lutalyse, Upjohn, Kalamazoo, MI) and ovarian follicular development was monitored by daily transrectal ultrasonography until ovariectomy. Animals were randomly assigned to one of two treatment groups: 1) the dominant follicle group (DF; $n=4$), or 2) the hCG-induced ovulatory follicle group (OF; $n=4$). In the DF group, the ovary bearing the dominant follicle on the morning of day 5 of the synchronized estrous cycle (day 0 = day of estrus) was obtained by ovariectomy. During the synchronized estrous cycle, the OF was obtained following an injection of 25 mg of $PGF_{2\alpha}$ (Lutalyse) at 22:00 h on day 7 to induce luteolysis, thereby converting the DF of the first follicular wave into an ovulatory follicle. An ovulatory dose of hCG (3000 IU, iv; APL, Ayerst Lab, Montréal, QC) was injected 36 h after the induction of luteolysis, and the ovary bearing the hCG-induced ovulatory follicle was collected by ovariectomy 24 h after hCG injection. Granulosa cells and follicular fluid were collected separately from individual follicles as described (Bédard et al., 2003). In the small follicle (SF) group, granulosa cells were collected from 2 to 4 mm follicles following their measurement, with calipers, at the surface of the ovary. Ovaries from Holstein cows were collected at the slaughterhouse in order to obtain a total of three pools of 20 small follicles (SF). Granulosa cells collected from SF ($n=20$) were pooled to generate sufficient amounts of total RNA. Corpus luteum at day 5 (D5) of the estrous cycle was obtained by ovariectomy as described above. Total RNA was isolated from granulosa cells or CL, quantified by optical density at 260 nm and quality was evaluated by electrophoretic separation as described (Bédard et al., 2003). These experiments were approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine of the Université de Montréal.

2.2. Quantitative RT-PCR of bovine VASAP-60 mRNA

The expression pattern of *VASAP-60* mRNA during final follicular development, ovulation and CL formation was analyzed by quantitative RT-PCR. Quantification of mRNA in granulosa cells of SF, DF or OF, and CL, was performed in a two-step real-time RT-PCR procedure using the fluorescent marker SYBR Green I and a LightCycler (Roche Applied Science, Laval, QC). The first step consisted of an RT reaction performed with 2 μg of total RNA per sample in the presence of oligo-dT and Omniscript (Qiagen Inc, Mississauga, ON) as described by the manufacturer. The second step consisted of a PCR amplification with specific primers for bovine *VASAP-60*