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Biological activities of tethered-eCG α -subunit COOH-terminal region

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Chorionic gonadotropin (CG) is synthesized and secreted by placenta trophoblast cells. CG is a member of the glycoprotein hormone family, which also includes luteinizing hormone (LH), follicle-stimulating hormone (FSH), human chorionic gonadotropin (hCG) and thyroid-stimulating hormone (TSH). These hormones consist of non-covalently associated α and β subunits. The α subunit is common to all glycoprotein hormones, whereas the β subunits have distinct sequences and confer receptor-binding specificities. Equine CG is a unique member of the gonadotropin family since it appears to be a single molecule that possesses both LH- and FSH-like activities in other animals.

We don't know why glycoprotein hormones have a common α subunit. Thus, our Lab. have been studying on the functions of tethered-eCGs in C-terminal region deletion (RDB, 2005 meeting, No, 2, 29: P0583). We performed the hormone and cAMP assays. The tethered-wt eCG, $\Delta 96$, $\Delta 95$ and $\Delta 93$ were efficiently secreted but not the $\Delta 87$. We checked the LH and FSH-like activities of these mutant eCGs. FSH-like activity, the $\Delta 95$ and $\Delta 93$ had very low activity, while the $\Delta 96$ was little activity compared to the wild-type eCG. LH-like activities, the $\Delta 95$ and $\Delta 93$ had very low activity, while $\Delta 96$ showed higher activity than wild-type eCG. We analyzed experiment *in vivo* with these mutants. Each mutant was injected with 2 IU to tail vein, and serum was collected at 10min, 30min, 2h and 4h. Each sample concentration in plasma was checked using the eCG ELISA kit. The result was peak at



10min in all treatment group, and it disappeared at 4h after injection. The wild-type concentration was lower than other mutants, but its pattern was similar. One of the interesting result was the identification of the eCG $\Delta 87$ and RNA transcription was checked by RT-PCR, Real-Time PCR and Northern blotting. We collected internal protein by cell membrane lysis in which the $\Delta 87$ sample was not detected in the cell lysate. These results imply a direct interaction between receptor and the COOH terminal region of the α -subunit. The α -subunit COOH terminal region was very important for secretion and function of hormone.

Keywords: *eCG, ELISA, cAMP, Northern blotting, pharmacokinetics*