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CO-LOCALIZATION OF LHβ AND FSHβ mRNAS IN THE PORCINE ANTERIOR PITUITARY BY IN SITU HYBRIDIZATION WITH BIOTINYLATED PROBES

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The localizations of mRNAs encoding LHβ and FSHβ in porcine pituitary were investigated by in situ hybridization technique. Biotinylated porcine LHβ and FSHβ cDNA probes were used on frozen sections of paraformaldehyde-fixed pituitary specimens. Hybridizations to both mRNAs were observed specifically in cytoplasm with unstained nuclei. Furthermore, cells hybridized for LHβ mRNA were demonstrated to be identical to those for FSHβ mRNA. This study provided the first morphological evidence that both gonadotropin β genes are expressed in the same cell. © 1988 Academic Press, Inc.

The glycoprotein hormone family in the pituitary is composed of three structurally related polypeptide hormones: LH, FSH, and TSH. Each of the three hormones consists of a common α-subunit and a different, biologically specific β-subunit for each hormone. By using antisera against β-subunits, immunocytochemical studies have shown that gonadotropic cells in pituitaries of many mammalian species synthesize both LH and FSH (1-5). Even some secretory granules in the same multihormonal cell contain both LH and FSH molecules (2).

In the present study, we applied in situ hybridization with two porcine cDNA probes encoding LHβ and FSHβ to demonstrate the localization of their respective messenger RNAs in the porcine anterior pituitary.

MATERIALS AND METHODS

Tissue preparation: Fresh porcine pituitaries taken from slaughterhouse were fixed in 4% neutral-buffered paraformaldehyde at 4°C for 24 hr, followed by overnight immersion in 15% sucrose

Abbreviations: NBT: nitro-blue tetrazolium.
BCIP: 5-bromo-chloro-3-indolyl phosphate.

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in PBS at 4°C. Tissue specimens were then embedded in OCT compound (Ames company) and frozen on dry ice. Adjacent 6-μm-thick sections were cut at -20°C in a cryostat, thaw-mounted on gelatinized glass slides and stored at -20°C until use.

Probes: Isolation and subclone preparation of two cDNA probes encoding porcine LHβ and FSHβ, respectively, have been described and no cross-hybridization was demonstrated between the two cDNA inserts (6, 7). Labeling of probes with biotin-11-dUTP (BRL) was carried out by multiprime labeling method (8) with reagents from an oligolabeling kit (Pharmacia).

In situ hybridization: The sections were preincubated for 2 hr at room temperature in 50% formamide, 1.2 M NaCl, 20 mM Tris-HCl buffer, pH 7.5, 2x Denhardt's solution (1x Denhardt's: 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 1 mM EDTA, 0.1% yeast total RNA, 0.01% tRNA and 0.1% denatured salmon sperm DNA. Hybridization was performed for 48 to 72 hr at room temperature in the above solution containing 10% dextran sulfate, 0.01% polyadenylitic acid and appropriately diluted, denatured, biotinylated probe. After hybridization, the slides were washed at room temperature in three 30-min changes with constant shaking in 50% formamide, 2x SSC (SSC: 150 mM NaCl, 15 mM Na citrate, pH 7.0), in 0.01% Triton X-100 , 2x SSC, and finally in 1x SSC.

Detection of the biotinylated probes: A biotinylated probe detection kit was used under the recommendation of the manufacturer (DNA detection system, BRL). Briefly, the sections were incubated with streptavidin diluted in 0.1 M Tris-HCl, pH 7.5, 0.1 M NaCl (buffer A) for 30 min at room temperature, then washed with 1x SSC for 5 min (three changes). The sections were then incubated with biotinylated alkaline phosphatase diluted in buffer A for 30 min and washed as above for two times, followed by 5 min wash in 0.1 M Tris-HCl, pH 9.5, 0.1 M NaCl and 50 mM $MgCl_2$. Dye solution containing NBT (nitro-blue tetrazolium) and BCIP (5-bromo-4-chloro-3-indolyl phosphate) was reacted with the sections for 2 to 4 hr in the dark. The color reaction was terminated by washing in 20 mM Tris-HCl, pH 7.5, 5 mM EDTA. The slides were mounted with glycerol and observed under light microscope.

Controls: Tissue sections were pretreated for 30 min with RNase A (10μg/ml)(Sigma) in 20 mM Tris-HCl, pH 7.6 before hybridization. A further control was carried out by adding denatured, unlabeled cDNA probe to the hybridization mixture.

RESULTS

Fig. 1A shows in situ hybridization study of the porcine anterior pituitary tissue when probed with cDNA complementary to LHβ mRNA. It is apparent that the hybridization signal was confined to the cytoplasm, while the nucleus remained unstained. The positive cells were often round or ovoid in shape. The hybridization product which contained grain-like structures was always seen around the nucleus. The numbers and intensities of the grains varied among the hybridization positive cells, probably reflecting the differences in the mRNA contents. Hybridization with FSHβ cDNA probe showed the same results as that with LHβ cDNA probe (figure not shown).

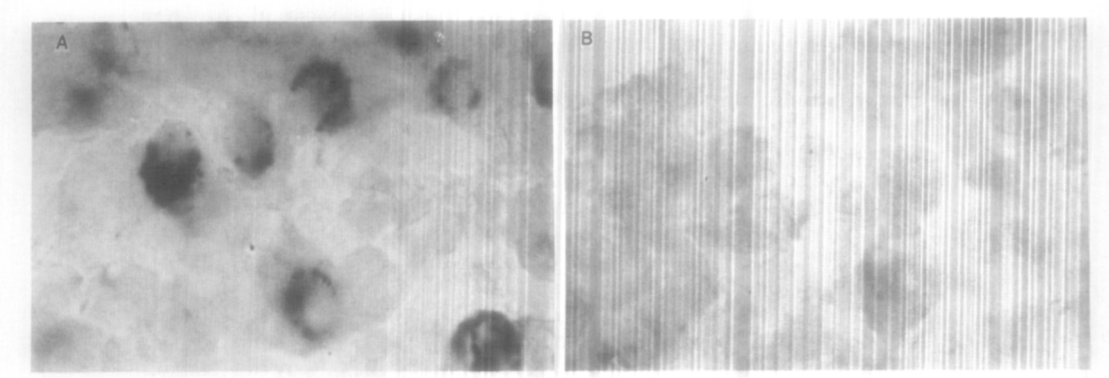

Fig. 1. In situ hybridization of LHβ mRNA with biotinylated LHβ cDNA probe in sections of the porcine anterior pituitary. (A). Hybridization positive cells are evident by the labeling restricted to the cytoplasm. Note the grain-like structures in the hybridization product. (B). Section pretreated with RNase before hybridization with the same probe shows no signal. X 360.

When the tissue section was pretreated with RNase, the hybridization signal was abolished, suggesting that the cDNA probe was hybridizing to RNA (Fig. 1B). No or only faint hybridization for LHβ or FSHβ mRNA was found, when excess amount of unlabeled probe was added to the hybridization buffer during the hybridization step (data not shown).

The co-localization of LHβ and FSHβ mRNA was investigated in adjacent sections (Fig. 2). The distribution of cells which contained LHβ or FSHβ mRNA was very similar. Indeed, cells that contained both LHβ and FSHβ mRNAs could be observed in the adjacent sections, indicating that one cell could express both LHβ and FSHβ genes. It was also shown that some cells in one

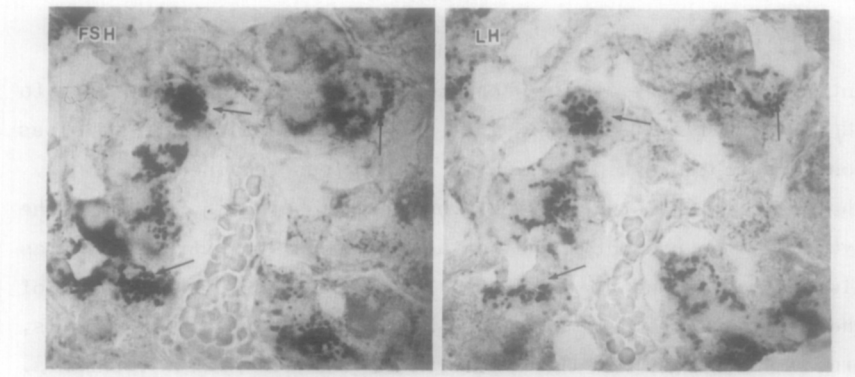

Fig. 2. Co-localization of FSHβ and LHβ mRNAs in adjacent sections. Cells containing both FSHβ and LHβ mRNAs are indicated by arrows. X 300.

section were positive for LHβ mRNA, but the identical cells did not hybridize for FSHβ mRNA in the adjacent section , or vice versa. This might be due to that the tissue was too thick to observe the same cell in two adjacent sections, although we still could not exclude the possibility that some cells contain only FSHβ or LHβ mRNA. The observations were reproducible upon repeated experiments.

DISCUSSION

Hybridization with biotinylated probes permits to visualize the localization of mRNAs in the porcine anterior pituitary. Biotinylated probe is superior to radio-labeled probe with respect to resolution and speed of process (10, 11). We tried ^{32}P- and ^{35}S-labeled probes in preliminary experiments, but the high background and the tendency of silver grains to extend outside the cytoplasm made it difficult to co-localize the hybridization positive cells in the adjacent sections (data not shown). The procedure with biotinylated probe results in good resolution which enables us to recognize the coexistence of LHβ and FSHβ mRNAs in adjacent sections. In preliminary experiments, we found that neither proteolytic digestion nor HCl treatment was necessary. We also omitted the acetylation step which was thought to reduce nonspecific reaction (9). Our present results suggest an promising approach for further in situ hybridization study.

The previous ultrastructural immunocytochemical study of porcine pituitary showed that most of the gonadotropes stained for both LH and FSH, while some were only positive for FSH or LH and that the concentrations of LH and FSH varied among the immunoreactive cells (5). Later, the same author demonstrated that in castrated pig all gonadotropes contained both LH and FSH suggesting that gonadectomy in pig increases the synthesis and storage of LH and FSH in all the gonadotropes (12). Although we could not exclude a possibility that cells containing only LHβ or FSHβ mRNA exist, our present observation that LHβ and FSHβ genes are expressed in the same cell extends our knowledge to the gene level beyond the results of the immunocytochemical studies. To our knowledge, this is the first report on the co-localization of LHβ and FSHβ mRNAs in the same cell of the anterior pituitary.

The regulation mechanism of nonparallel release of LH and FSH has been of great concern because of its important role in the

maintenance of normal estrous cycle. Study on the α and LHβ mRNAs changes during the rat estrous cycle showed that α and LHβ gene expression is coordinately regulated except for the pro-estrous phase, in which the LHβ gene can be preferentially expressed (13). By using our established in situ hybridization method, further study on the cellular variations in the levels of LHβ and FSHβ gene transcription within the pituitary sections could give more informations on the molecular basis of differential LH and FSH release from the same gonadotropes under different physiological conditions.

In conclusion, the present in situ hybridization study with biotinylated probes illustrates the precise morphological localizations of LHβ and FSHβ mRNAs in the porcine pituitary gland. The co-localization of LHβ and FSHβ mRNAs is demonstrated in adjacent sections, indicating that the two genes are expressed in the same cell.

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