AMP-activated protein kinase activation reduces the transcriptional activity of the murine luteinizing hormone β-subunit gene

Ryutaro MORIYAMA1), Koichi IWAMOTO1), Teruki HAGIWARA2), Saishu YOSHIDA3, 4), Takako KATO4) and Yukio KATO4)

1)Laboratory of Environmental Physiology, Department of Life Science, School of Science and Engineering, Kindai University, Osaka 577-8502, Japan
2)Laboratory of Molecular and Cellular Biology, Department of Life Science, School of Science and Engineering, Kindai University, Osaka 577-8502, Japan
3)Department of Biochemistry, The Jikei University School of Medicine, Tokyo 105-8461, Japan
4)Laboratory of Molecular Biology and Gene Regulation, Department of Life Science, School of Agriculture, Meiji University, Kanagawa 214-8571, Japan

Abstract. Malnutrition is one of the factors that induces reproductive disorders. However, the underlying biological processes are unclear. AMP-activated protein kinase (AMPK) is an enzyme that plays crucial role as a cellular energy sensor. In the present study, we examined the effects of AMPK activation on the transcription of the murine gonadotropin subunit genes Cga, Lhb, and Fshb, and the gonadotropin-releasing hormone receptor Gnrh-r. Real-time PCR and transcription assay using LβT2 cells demonstrated that 5-amino-imidazole carboxamide riboside (AICAR), a cell-permeable AMP analog, repressed the expression of Lhb. Next, we examined deletion mutants of the upstream region of Lhb and found that the upstream regulatory region of Lhb (–2527 to –2198 b) was responsible for the repression by AICAR. Furthermore, putative transcription factors (SP1, STAT5a, and TEF) that might mediate transcriptional control of the Lhb repression induced by AICAR were identified. In addition, it was confirmed that both AICAR and a competitive inhibitor of glucose metabolism, 2-deoxy-D-glucose, induced AMPK phosphorylation in LβT2 cells. Therefore, the upstream region of Lhb is one of the target sites for glucoprivation inducing AMPK activation. In addition, AMPK plays a role in repressing Lhb expression through the distal –2527 to –2198 b region.

Key words: 5-Amino-imidazole carboxamide riboside (AICAR), AMP-activated protein kinase (AMPK), Glucoprivation, Luteinizing hormone (LH), Pituitary

Received: November 7, 2019
Accepted: November 29, 2019
Advanced Epub: December 9, 2019
©2020 by the Society for Reproduction and Development
Correspondence: R. Moriyama (e-mail: mory@life.kindai.ac.jp)
This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License. (CC-BY-NC-ND 4.0: https://creativecommons.org/licenses/by-nc-nd/4.0/)
to be an intracellular sensor that is activated by an energy deficiency, such as hypoglycemia, or by several hormones that are secreted during malnutrition. AMPK plays a pivotal role in the regulation of peripheral energy homeostasis, since AMPK is activated by the intracellular AMP/ATP ratio when ATP levels decrease [12]. Therefore, AMPK might be involved in reproductive control as a sensor of the peripheral energy status at several points along the H-P-G axis [13]. AMPK stimulation inhibits LH and FSH secretion at the pituitary level, and LH secretion in LβT2 cells [3]. However, the effect of AMPK on the response elements of gonadotropin subunit genes at the pituitary level is not well understood.

This study examined whether intracellular energy depletion regulates the transcription of the murine gonadotropin subunit genes Cga, Lhb, Fshb, and Gnrh-r via AMPK activation, and sought to confirm the gene regulatory region that is responsive to AMPK activation in vitro. We used 5-amino-imidazole carboxamide riboside (AICAR), a cell-permeable AMP analog, to mimic intracellular energy depletion [15].

Materials and Methods

Cell cultures

The LβT2 mouse gonadotropic cell line provided by Dr PL Mellon (Department of Reproductive Medicine, University of California, San Diego, CA, USA) was maintained in monolayer cultures in Dulbecco’s modified Eagle’s medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum and 0.5% penicillin/streptomycin solution (Sigma-Aldrich, St. Louis, MO, USA) in a humidified 5% CO₂/95% air incubator at 37°C. For transfection experiments, LβT2 cells were exposed to 50, 100, or 200 μM AICAR for 48 h. AICAR treatment

AICAR (Sigma-Aldrich) was dissolved in water and the 250 mM stock solution was stored at –20°C until use.

RNA extraction and cDNA synthesis

Total RNA was prepared from LβT2 cells and pituitary glands of decapitated mice using TRI Reagent (Sigma-Aldrich) and treated with RNase-free DNase I to remove any genomic DNA contamination. Then, cDNA was synthesized using a Superscript IV kit with an oligo(dT)12-18 primer. All reagents were purchased from Thermo Fisher Scientific.

Real-time PCR

The mRNA levels of Lhb, Fshb, Cga, and Gnrh-r in LβT2 cells were determined by real-time PCR using SYBR Premix Ex Taq II (TaKaRa Bio, Shiga, Japan) containing SYBR Green I, in a 7500 Real-time PCR System (Applied Biosystems, Foster City, CA, USA). The following conditions were used: denaturation at 95°C for 30 sec and amplification by cycling 40 times at 95°C for 5 sec and at 60°C for 34 sec. Data were analyzed using the standard curve method and normalized to TATA-box binding protein (Tbp) expression as the reference gene. The forward and reverse primer sets (Thermo Fisher Scientific) used for each gene are shown in Table 1. To test the effect of AICAR over the expression of tested genes, in some experiments LβT2 cells were exposed to 50, 100, or 200 μM AICAR for 48 h.

RT-PCR

The expression of the transcription factor genes was determined by RT-PCR. cDNAs were amplified by PCR using GoTaq DNA polymerase (Promega, Madison, WI, USA). The cycling protocol used was as follows: an initial denaturation step for 60 sec at 94°C;
35 cycles of denaturation for 30 sec at 94°C, primer annealing for 30 sec at 56°C, and extension for 90 sec at 72°C; and 7 min at 72°C at the end of cycling to complete extension. The amplified products were then separated on 2% agarose gels containing 0.5 μg/ml ethidium bromide. The forward and reverse primers (Thermo Fisher Scientific) used are shown in Table 1.

**Harr-plot analysis**

Harr-plot analysis is a graphical method that allows the comparison of two nucleotide sequences and identification of regions of similarity between them. Nucleotide sequence homology was determined between the mouse and rat 5′-flanking regions, up to 4.0 kb from the transcription initiation sites of the Cga gene and up to 3.0 kb from the transcription initiation sites of Lhb and Fshb genes. Each dot represents > 85% identity in 20 nucleotides.

**Reporter assay**

Upstream regions of the rat Cga (NC_005104.4), Fshb (NC_005102.4), and Lhb (NC_005100.4) genes were amplified using specific primer sets. Fragments were ligated into the secreted alkaline phosphatase (SEAP) plasmid vector pSEAP2-Basic (Clontech Laboratories, Palo Alto, CA, USA) as described previously [16, 17]. Resulting reporter vectors contained the following gonadotropin subunit upstream regions: –3793 to +37 of Cga; –2824 to +28 of Fshb; and –2930 to +17, –2527 to +17, –2197 to +17, –1976 to +17, –1595 to +17, –1370 to +17, –1097 to +17, and –433 to +17 of Lhb.

Transfection was performed using 200 ng of DNA and 0.3 μl FuGENE HD (Roche Diagnostics, Basel, Switzerland) per well according to a protocol described in a previous study [18]. Cells were treated with an AICAR solution (10 μl per well) 7 to 8 h after transfection and incubated for 48 h. Then, 5 μl of culture medium from each well was assayed for SEAP activity using a Phospha-Light Reporter Gene Assay System (Applied Biosystems) and a Powerscan H1 microplate luminometer (DS Pharma Biomedical, Osaka, Japan) according to the manufacturers’ protocols.

**Western blotting**

Overnight serum-starved LβT2 cells were treated either with 25 mM 2-deoxy-D-glucose (2DG) for 0.5, 1 or 4 h, or with 100 or 200 μM AICAR for 24 h and then lysed in extraction buffer (50 mM Tris-Cl, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100) containing 1% protease inhibitors (Nakarai Tesque, Kyoto, Japan) and a phosphatase inhibitor cocktail (Sigma-Aldrich). Total cell lysates were centrifuged at 15000 g for 5 min. Supernatants were mixed with 4 × sodium dodecyl sulfate sample buffer, boiled, and separated in polyacrylamide gels. Proteins were then transferred to polyvinylidene difluoride membranes (Millipore, San Jose, CA, USA). The membranes were probed with primary antibodies at the following dilutions: anti-AMPKα polyclonal antibody (1:1000; Cell Signaling Technology, Danvers, MA, USA), anti-phospho-AMPKα (Thr172) polyclonal antibody (1:100; Cell Signaling Technology), or anti-β-actin monoclonal antibody (1:2000; Sigma-Aldrich). Membranes were further incubated with anti-rabbit IgG antibody conjugated to horseradish peroxidase (HRP) (1:4000, Cell Signaling Technology) or HRP-conjugated anti-mouse IgG antibody (1:2000, Santa Cruz Biotechnology, Dallas, TX, USA), and developed with ImmunoStar Zeta (Fujiﬁlm Wako Chemicals, Osaka, Japan). Chemiluminescence was recorded using an ImageQuant LAS 500 (GE Healthcare, Chicago, IL, USA).

**Microarray analysis**

Total RNAs were prepared from the whole pituitaries of embryonic day (E)12.5 (n = 26), E13.5 (n = 15), E14.5 (n = 10), E16.5 (n = 14), E18.5 (n = 11), E20.5 (n = 10), and postnatal day (P)0 (n = 9) rats, and from the anterior lobes of P5 (n = 9), P15 (n = 8), P30 (n = 4), P60 (n = 3), and P600 (n = 3) rats using ISOGEN (Nippon Gene, Tokyo, Japan). Microarrays were performed using Rat Genome 230 2.0 GeneChip (Affymetrix Japan, Tokyo, Japan) for total RNA samples. Data of microarrays were normalized by median normalization.

**Statistical analyses**

All values are expressed as the mean ± standard error of the mean (SEM). One-way ANOVA followed by Dunnett’s multiple comparison test was used to analyze the effect of AICAR on SEAP activity. P-values < 0.05 were considered significant.

**Results**

**Effect of AICAR on mRNA expression levels of mouse gonadotropin subunit genes and Gnrh-r**

After 48 h of treatment, AICAR induced significant decreases in Lhb mRNA levels in LβT2 cells (Fig. 1). In contrast, mRNA levels of Fshb, Cga, and Gnrh-r were not significantly different.

**Homology sequence between the mouse and rat 5′-flanking regions of gonadotropin subunit genes**

Harr-plot analyses were performed to confirm the sequence homology between the mouse and rat 5′-flanking regions of the Cga, Lhb, and Fshb genes, since a reporter assay was performed using each rat gene in the mouse pituitary-lineage cell line, LβT2. At a maximum upstream region of 4.0 kb from the transcription initiation site of Cga gene and 3.0 kb from that of Lhb and Fshb genes, mice and rats shared a high degree of identity in their nucleotide sequences (Fig. 2).

**Effect of AICAR on the transcriptional activation of rat gonadotropin subunit genes**

The promoter activity of rat Lhb (–2930 to +17) was significantly repressed by treatment with 100 and 200 μM AICAR (P < 0.05; Fig. 3). Conversely, the promoter activities of rat Cga (–3793 to +37 bp) and Fshb (–2824 to +28) genes were not significantly repressed by either AICAR concentration. Furthermore, 50 μM of AICAR treatment did not affect the promoter activity of rat Cga (–3793 to +37), Lhb (–2930 to +17), and Fshb (–2824 to +28).

**Deletion analysis for the Lhb upstream region**

To confirm the repression of Lhb by 100 μM AICAR, deletion mutants of the upstream region of Lhb were examined using transfection and reporter gene assays. AICAR significantly repressed the promoter activity of the –2930 to +17 and the –2527 to +17 regions (Fig. 4). However, the –2197 to +17, –1976 to +17, –1595 to +17, –1370 to +17, –1097 to +17, –718 to +17, and –433 to +17 regions were not significantly affected.
were not significantly repressed by AICAR.

**Nucleotide sequence homology between –2527 and –2198 b promoter regions of the rat and mouse Lhb genes**

The nucleotide sequences of the –2527 to –2198 b regions upstream of the mouse and rat Lhb genes shared 90.3% homology (Fig. 5a). Fig. 5a and 5b show the locations of putative binding sites for transcription factors, along the –2527 to –2198 b regions of both the mouse and rat Lhb genes that responded to AICAR induced AMPK activation. Among the putative transcription factors that bind to these sites, activating enhancer binding protein 2α (AP-2α), specificity protein 1 (SP1), signal transducer and activator of transcription 1 (STAT1), signal transducer and activator of transcription 5A (STAT5a), transcription factor 3 (TCF3), and thyrotroph embryonic factor (TEF) were identified as those expressed in the rodent pituitary gland. Table 2 shows the expression pattern of these transcription factors in the rat pituitary examined by microarray analysis. All these transcription factors, except AP-2α, were constitutively expressed in the rat pituitary gland from fetal to adult stages.

**Effect of AICAR on mRNA expression levels of transcription factors that bind to the Lhb upstream region in LβT2 cells**

Mouse Sp1, Stat1, Stat5a, Tcf3, and Tef mRNA expression was detected by RT-PCR (Fig. 6a) in mouse pituitary and LβT2 cells, whereas AP-2α expression could not be found. After 48 h of exposure to either 100 or 200 μM AICAR, mRNA levels of mouse Sp1, Stat5a, and Tef, but not Stat1 and Tcf3, tended to decrease, although not significantly, in contrast with those from untreated LβT2 cells (Fig. 6b).

**Effect of 2DG or AICAR on AMPK activation in LβT2 cells**

Glucoprivation induced by 25 mM 2DG enhanced the phosphorylation of AMPK (Thr172) in LβT2 cells (Fig. 7). Furthermore, artificial AMPK activation by 0.1 or 0.2 mM AICAR, an AMP analog, also enhanced phosphorylation of AMPK (Thr172). Both 2DG and AICAR induced a significant increase in phosphorylated AMPK.
AMPK REDUCES Lhb TRANSCRIPTION

Discussion

The present study demonstrated that AICAR down-regulated Lhb transcription in LβT2 cells. The result indicates that the AMPK signaling pathway directly or indirectly inhibits Lhb transcription, to regulate the H-P-G axis at the pituitary level. This inhibition seems to be independent of GnRH-R, because AICAR did not alter the mRNA level of Gnrh-r in this study. Considering that protein synthesis requires the recruitment of mRNA, it would have a close correlation between transcription and translation rates in general. Thus, the AMPK signaling pathway might down-regulate not only Lhb transcription but also LHβ protein translation and subsequent LH synthesis in LβT2 cells. In fact, it has been reported that AICAR induces not only suppression of Lhb transcription but also LH secretion in LβT2 cells [3]. On the other hand, FSH would ordinarily be synthesized in sufficient quantity in LβT2 cells despite the administration of AICAR, given the abundant Cga. In addition, the regulatory mechanism of transcription of gonadotropin hormone subunit genes induced by AMPK activation may be at least partly similar or the same among murine species, especially between rats and mice, because rats and mice share a high degree of identity in their nucleotide sequences in 5′-flanking regions until approximately 4.0 kb upstream from the transcription initiation site of Cga gene and approximately 3.0 kb of Lhb and Fshb genes. Indeed, the results from the mouse mRNA expression assay were almost the same as those from the rat promoter assay in this study. Tosca et al. [14] reported

Fig. 3. Transient transfection assay of rat gonadotropin subunit gene promoters with or without treatment with the AMP-activated protein kinase (AMPK) activator 5-amino-imidazole carboxamide riboside (AICAR) in LβT2 cells. Reporter constructs containing Cga (−3793 to +37), Lhb (−2930 to +17), or Fshb (−2824 to +28) promoters fused with the secreted alkaline phosphatase (SEAP) gene in the pSEAP-Basic vector were transfected into LβT2 cells. The cells were exposed to 50, 100, or 200 µM AICAR. The reported activities are presented as the SEAP activity relative to that of the basic vector. Values are the mean ± SEM of four independent experiments. * P < 0.05 vs. pSEAP2-Basic.

Fig. 4. Deletion analysis of the rat luteinizing hormone β-subunit (Lhb) promoter region (−2930 to +17 b regions) with or without treatment with the AMP-activated protein kinase (AMPK) activator 5-amino-imidazole carboxamide riboside (AICAR) in LβT2 cells. The left portion shows reporter constructs containing serial deletion mutants of the Lhb promoter fused to the secreted alkaline phosphatase (SEAP) gene in the pSEAP2-Basic vector that were transfected into LβT2 cells. The right portion shows SEAP activities relative to that of the basic vector. Values are the mean ± SEM for four independent experiments. * P < 0.05 vs. pSEAP2-Basic.
Fig. 5. Nucleotide sequence alignment (a) and location of gene transcription factor binding sites (a, b) in the –2527 to –2198 b region upstream of the \textit{Lhb} promoter that was responsive to the AMP-activated protein kinase (AMPK) activator 5-amino-imidazole carboxamide riboside (AICAR). The rat luteinizing hormone \(\beta\)-subunit (\textit{Lhb}) promoter was compared with that of the mouse. Ap-2\(\alpha\), Activating enhancer binding protein 2\(\alpha\); SP1, specificity protein 1; STAT1, signal transducer and activator of transcription 1; STAT5A, signal transducer and activator of transcription 5A; TCF3, transcription factor 3; TEF, thyrotroph embryonic factor.

Table 2. Expression pattern of transcription factor genes that can bind to upstream site (–2527/–2198) of rat luteinizing hormone \(\beta\)-subunit (\textit{Lhb}) in the rat pituitary.

| Gene title | Gene symbol | E12.5 | E13.5 | E14.5 | E16.5 | E18.5 | E20.5 | P0 | P5 | P15 | P30 | P60 | P600 |
|------------|-------------|-------|-------|-------|-------|-------|-------|----|----|-----|-----|-----|-----|
| Activating enhancer binding protein-2, alpha | Ap-2\(\alpha\) | 0     | 0     | 0     | 0     | 0     | 0     | 0  | 0  | 0   | 0   | 0   | 0   |
| Specificity protein 1 | Spi | 11    | 12    | 11    | 9     | 9     | 8     | 8  | 7  | 7   | 7   | 7   | 7   |
| Signal transducer and activator of transcription 1 | Stat1 | 3     | 3     | 4     | 4     | 5     | 6     | 7  | 10 | 12  | 14  | 11  |     |
| Signal transducer and activator of transcription 5A | Stat5A | 1     | 2     | 2     | 1     | 1     | 1     | 2  | 1  | 2   | 2   | 2   |     |
| Transcription factor 3 | Tcf3 | 5     | 4     | 4     | 2     | 2     | 2     | 2  | 1  | 1   | 1   | 1   | 1   |
| Thyrotroph embryonic factor | Tef | 2     | 1     | 2     | 2     | 3     | 3     | 4  | 5  | 5   | 4   | 3   | 5   |
that metformin, an indirect activator of AMPK, inhibited not only Lhb mRNA expression but also Fshb in rat pituitary cell cultures. The different in results between the prior and the present studies may reflect the different cell types and drugs used. In the present study, we showed the effect of AICAR on a mouse gonadotrope cell line. Cell lines often alter their phenotype, native functions, and their responsiveness to stimuli [19]. Indeed, the altered responsiveness to stimuli between gonadotrope and rodent pituitary cell cultures was previously reported [20]. AICAR is recognized as an AMP mimetic activator of the AMPK that directly activates AMPK [15], whereas metformin is recognized as an indirect activator of the AMPK that induces AMPK activation through inhibition of complex I of the respiratory chain in mitochondria [21]. These differences in cell types and drugs would result in a different outcome. Therefore, it is necessary to keep in mind that the murine gonadotropin transcription control mechanism under the physiological condition may differ somewhat from the present results. Indeed, the present study showed that the promoter activities of Fshb tended to be repressed. The results concerning the AMPK response in the transcription of the murine gonadotropin hormone subunit gene are still not conclusive. The –2527 to −2198 b region of the Lhb gene was identified as a novel region for the transcriptional control by AMPK. This region may contain several putative regulatory elements for diverse transcription factors, including SP1, STAT1, STAT5a, TCF3, and TEF. The previous studies showed that AMPK activation decreases the protein level of SP1 and STAT1 in many types of cells [22–25]. In this study, however, we observed the decreasing trend in Sp1, Stat5a, and Tef mRNA, but not in Stat1. Therefore, further investigations of the suppression by AMPK activation should clarify the role of molecules and their mechanisms through the –2527 to −2198 bp upstream region of the Lhb gene.

Metabolic disorders suppress pulsatile LH secretion in mammals

**Fig. 6.** a) Gene expression, determined by RT-PCR, of the transcription factors, activating enhancer binding protein 2α (Ap-2α), specificity protein 1 (Sp1), signal transducer and activator of transcription 1 (Stat1), signal transducer and activator of transcription 5A (Stat5a), transcription factor 3 (Tcf3), and thyrotroph embryonic factor (Tef), that might bind to the –2527 to −2198 b region upstream of the rat luteinizing hormone β-subunit (Lhb) promoter in mouse and LβT2 cells. b) Real-time PCR of the same transcription factors, except for Ap-2α, after a 48-h treatment with 100 or 200 μM 5-amino-imidazole carboxamide riboside (AICAR). Data are expressed as the mean ± SEM for four independent experiments. Each mRNA value was normalized using the TATA-box binding protein (Tbp) mRNA level as a reference. * P < 0.05 vs. 0 μM AICAR (control).

**Fig. 7.** AMP-activated protein kinase (AMPK) phosphorylation is stimulated by 2-deoxy-D-glucose (2DG) and 5-amino-imidazole carboxamide riboside (AICAR). LβT2 cells were treated with either 25 mM 2DG for 0.5, 1, or 4 h, or with 100 or 200 μM AICAR for 24 h. Total cell lysates were extracted and subjected to immunoblotting using anti-phospho-AMPKα (Thr172), anti-AMPKα, and anti-β-actin antibodies. Densitometric analysis was performed in three experiments, and phospho-AMPKα (pAMPK) was normalized for β-actin. Data are expressed as the mean ± SEM. * P < 0.05 vs. Vehicle.
Several studies have also reported that an energy sensor regulating the H-P-G axis exists in the brain [29–31]. AMPK is recognized as a fuel gauge that is activated in response to fasting or glucoprivation. Presently, 2DG-induced glucoprivation induced AMPK phosphorylation in LβT2 cells. This result suggests that mouse gonadotropes directly sense glucoprivation to regulate the H-P-G axis. Furthermore, we previously described that LβT2 cells directly responded to long-chain fatty acid levels to regulate the transcription of gonadotropic hormones [5] and the expression of the long-chain fatty acid receptor GPR120 in mouse pituitary gonadotropes [32]. Thus, gonadotropes may sense not only blood glucose levels but also peripheral free fatty acid levels in the pituitary gland. Lu et al. [3] reported that adiponectin decreased LH secretion in the pituitary gonadotropes in an AMPK-dependent manner. Therefore, both the synthesis and secretion of gonadotropic hormones may be directly regulated by peripheral signals, such as hormones and nutrition, at the pituitary level.

In conclusion, the present study reveals that the transcription of the murine Lhb gene is inhibited by AMPK activation. Furthermore, AMPK repressive regulation of the murine Lhb gene expression involves the 5′-flanking region between –2527 and –2198 b.

Acknowledgments

We thank Dr PL. Mellon for providing us with the LβT2 cell line. This work was supported in part by the Japan Society for the Promotion of Science KAKENHI Grants 23780282 and 19K06446, and by a Kindai University grant RKS28-057 awarded to RM.

References

1. Pierce BN, Stackpole CA, Breen KM, Clarke LJ, Karoch FJ, Rivaland ET, Turner AI, Caddy DJ, Wengeraker ER, Oakley AE, Tilbrook AJ. Estradiol enables cortisol to act directly upon the pituitary to suppress pituitary Responsiveness to GnRH in sheep. Neuroendocrinology 2009; 89: 86–97. [Medline] [CrossRef]
2. Rodriguez-Pacheco F, Martinez-Fuentes AJ, Tovar S, Pinilla L, Tena-Sempere M, Dieguez C, Castaño JP, Malagón MM. Regulation of pituitary cell function by adiponectin. Endocrinology 2007; 148: 401–410. [Medline] [CrossRef]
3. Lu M, Tang Q, Olefsky JM, Mellon PL, Webster NJ. Adiponectin activates adenosine monophosphate-activated protein kinase and decreases luteinizing hormone secretion in LbetaT2 gonadotropes. Mol Endocrinol 2008; 22: 760–771. [Medline] [CrossRef]
4. Avellan-Cruz JE, Flores A, Cebada J, Mellon PL, Felix R, Monjaraz E. Leptin increases i-type Ca2+ channel expression and GnRH-stimulated LH release in LbetaT2 gonadotropes. Mol Cell Endocrinol 2009; 299: 55–65. [Medline] [CrossRef]
5. Moriarty R, Yamaizaki T, Kato T, Kato Y. Long-chain unsaturated fatty acids reduce the transcriptional activity of the rat follicle-stimulating hormone β-subunit gene. J Reprod Dev 2016; 62: 195–199. [Medline] [CrossRef]
6. Suzuki MI, Balkin DM, Chen Y, Woodruff TK. Smad3 mediates activin-induced transcription of follicle-stimulating hormone beta-subunit gene. Mol Endocrinol 2005; 19: 1849–1858. [Medline] [CrossRef]
7. Ciccone NA, Laczka CT, Hou MY, Gregory SJ, Kam KY, Xu S, Kaiser UB. A composite element that binds basic helix loop helix and basic leucine zipper transcription factors is important for gonadotropin-releasing hormone regulation of the follicle-stimulating hormone beta gene. Mol Endocrinol 2008; 22: 1908–1923. [Medline] [CrossRef]
8. Melamed P, Savelaesa D, Lim S, Wijewrera A, Luo Z, Luo M, Pauwel L. Gonadotro-phin-releasing hormone signalling downstream of calmodulin. J Neuroendocrinol 2012; 24: 1463–1475. [Medline] [CrossRef]
9. Fortin J, Ongaro L, Li Y, Tran S, Lamba P, Wang Y, Zhou X, Bernard DJ. Minireview: Activin Signaling in Gonadotropes: What Does the FOX Say… to the SMADs? Mol Endocrinol 2015; 29: 963–977. [Medline] [CrossRef]
10. Yoshida S, Kato T, Nishimura N, Kanno N, Chen M, Ueharu H, Nishihara H, Kato Y. Transcription of follicle-stimulating hormone subunit genes is modulated by porcine LIM homeobox transcription factors, LHX2 and LHX3. J Reprod Dev 2016; 62: 241–248. [Medline] [CrossRef]