Molecular Cloning and Identification of the Transcriptional Regulatory Domain of the Goat Neurokinin B Gene TAC3

Yuta SUETOMI1)*, Fuko MATSUDA1)*, Yoshihisa UENOYAMA2), Kei-ichiro MAEDA3), Hiroko TSUKAMURA2) and Satoshi OHKURA1)

1)Laboratory of Animal Production Science, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan
2)Laboratory of Reproductive Science, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan
3)Laboratory of Animal Breeding, Department of Veterinary Medical Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan

Abstract. Neurokinin B (NKB), encoded by TAC3, is thought to be an important accelerator of pulsatile gonadotropin-releasing hormone release. This study aimed to clarify the transcriptional regulatory mechanism of goat TAC3. First, we determined the full-length mRNA sequence of goat TAC3 from the hypothalamus to be 820 b, including a 381 b coding region, with the putative transcription start site located 143-b upstream of the start codon. The deduced amino acid sequence of NKB, which is produced from preproNKB, was completely conserved among goat, cattle, and human. Next, we cloned 5'-upstream region of goat TAC3 up to 3400 b from the translation initiation site, and this region was highly homologous with cattle TAC3 (89%). We used this goat TAC3 5'-upstream region to perform luciferase assays. We created a luciferase reporter vector containing DNA constructs from –2706, –1837, –834, –335, or –197 to +166 bp (the putative transcription start site was designated as +1) of goat TAC3 and these were transiently transfected into mouse hypothalamus-derived N7 cells and human neuroblastoma-derived SK-N-AS cells. The luciferase activity gradually increased with the deletion of the 5'-upstream region, suggesting that the transcriptional suppressive region is located between –2706 and –336 bp and that the core promoter exists downstream of –197 bp. Estradiol treatment did not lead to significant suppression of luciferase activity of any constructs, suggesting the existence of other factor(s) that regulate goat TAC3 transcription.

Key words: Estrogen, GnRH, Neurokinin B, Promoter, Ruminant

In mammalian females, two modes of gonadotropin-releasing hormone (GnRH) release regulate ovarian function. One is the pulsatile GnRH secretion that causes pulsatile luteinizing hormone (LH) and follicle-stimulating hormone (FSH) releases, which are responsible for follicular development and steroidogenesis [1–3]. The other is the surge mode of GnRH secretion followed by an LH surge, leading to ovulation. Both GnRH/LH pulse and GnRH/LH surge are controlled by estrogen feedback, but estrogen acts differently on these two secretory patterns: GnRH/LH pulses are negatively regulated by estrogen, while GnRH/LH surge is positively regulated by estrogen [4].

The question of which neuronal substrates are the target of estrogen feedback effects on GnRH secretion is still open to debate. Since GnRH neurons do not express estrogen receptor α (ERα) [5], other sites of estrogen action have been predicted. Currently, kisspeptin neurons, which express ERα [6, 7], are the major candidates to transfer estrogen signals to GnRH neurons and organize GnRH secretion [8]. Kisspeptin neurons are located in two regions of the hypothalamus, the anterior hypothalamus, such as the medial preoptic area (mPOA) and anteroventral periventricular nucleus (AVP), and the arcuate nucleus (ARC) [9–12]. Multiple lines of evidence have suggested that kisspeptin neurons in the ARC and the mPOA regulate GnRH/LH pulses and GnRH/LH surges, respectively [13]. Kisspeptin neurons in the ARC are called KNDy neurons since they co-express the neuropeptides neurokinin B (NKB) and dynorphin A (Dyn) in many species including goat [14–19], while kisspeptin neurons in the mPOA/AVP do not express NKB or Dyn; thus, NKB and Dyn are possible key molecules that make KNDy neurons to be the generator of GnRH pulses.

NKB, a member of the tachykinin family, is produced from preproNKB which is encoded by TAC3 gene in goat, cattle, and human [20]. NKB acts through the receptor NK3R, encoded by TACR3 gene. The loss-of-function mutants for TAC3 or TACR3 in humans exhibit gonadal dysfunction and the disappearance of normal LH release [21, 22], indicating a key role of NKB in reproductive function. Recent studies reported that an intracerebroventricular administration of NKB induced GnRH pulses in goats [18], and LH pulses were suppressed by the intravenous and intracerebroventricular injection of NKB receptor antagonist in monkeys and rats, respectively [17, 23]. On the other hand, Dyn administration gradually decreased LH concentrations in goats [18]. These data suggest that NKB functions...
Animals and sample preparation

Enhance/suppress increase/decrease NKB expression and intracellular molecules that GnRH/LH pulses is well known, the regulatory mechanism of NKB when using goats as an animal model. Therefore, we first identified experimental results if we apply the sequence information of goat system as described above and because we can obtain more precise logical study including the study of the GnRH/LH pulse generation those in goat because goat is a useful animal model for endocrinology. However, it is worth determining in other species including cattle and human TAC3 mRNA sequence and the 5'-upstream mechanism of goat.

The present study aimed to clarify the transcriptional regulatory mechanism of goat TAC3. The mRNA sequence and the 5'-upstream genomic sequence of TAC3 in other species including cattle and human have already been determined. However, it is worth determining those in goat because a useful animal model for endocrinological study including the study of the GnRH/LH pulse generation system as described above and because we can obtain more precise experimental results if we apply the sequence information of goat when using goats as an animal model. Therefore, we first identified the full-length mRNA sequence of goat TAC3 and the genomic sequence of the goat TAC3 5'-upstream region. Next, we evaluated the promoter activity of this region by luciferase assay using N7, immortalized mouse hypothalamic neural cells, and SK-N-AS cells, human neuroblastoma cells. Further, we examined the effect of estrogen on goat TAC3 promoter activity.

Materials and Methods

Shiba goats were used for this experiment. They were maintained under natural conditions at the Field Science Center, Graduate School of Bioagricultural Sciences, Nagoya University, Japan. Goats were fed twice a day with free access to water and supplemental minerals.

To prepare total RNA for full-length mRNA analysis, the ARC tissue was obtained from the hypothalamus of a 3-week-old male goat after injection with an overdose of sodium pentobarbital. To prepare genomic DNA for determining goat TAC3 5'-upstream sequence, a 2 ml blood sample was collected from a 7-month-old female goat. All experiments were approved by the Committee on Care and Use of Experimental Animals of the Graduate School of Bioagricultural Sciences, Nagoya University.

Determination of goat TAC3 5'-upstream sequence

The collected ARC tissue was homogenized and total RNA was extracted with TriPure Isolation Reagent (Roche Diagnostics, Basel, Switzerland) following the manufacturer's protocol, and then 5'-rapid amplification of cDNA ends (RACE) and 3'-RACE methods were performed using GeneRacer Kits (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions. The reverse gene-specific primer used for 5'-RACE was 5'-ACCAC CAGGA TGAGC AAGAT CCTAT CCCA-3' and 5'-ATGAT TGGGA GGTGA GTCTG GCACC TCT-3' for the second round and 5'-CTTGG GATTT GGAAG AGATA CATAG CTCCTG-3' and 5'- AATGG TTCTCT GCTCA CCAGC ACCTT TCCCA-3' for the third round. The predicted transcription factor binding site in the obtained goat TAC3 5'-upstream region was analyzed using the Transcriptional Element Search System (TESS) program (http://www.cbil.upenn.edu/tess). The sequence of the goat TAC3 5'-upstream region was compared with those of cattle and human TAC3 using ClustalW. The 5'-upstream sequences of cattle and human TAC3 were obtained from the University of California, Santa Cruz (UCSC) genome bioinformatics website (http://genome.ucsc.edu/).

Construction of luciferase reporter vectors

To construct the reporter vector for luciferase assay, the 5'-fragment of goat TAC3 containing sequence –2706, –1837, –834, –335, or –197 to +166 were amplified by genomic PCR with primers including XhoI and HindIII sites at the 5' and 3' ends, respectively. Each amplified fragment was digested by XhoI and HindIII, and was inserted into a luciferase reporter vector, pGL4-basic (Promega, Madison, WI, USA). The constructed plasmids were designated as pGL-2706, pGL-1837, pGL-834, pGL-335, and pGL-197 according to the respective positions of the fragments.

Transient transfection and luciferase assay

Luciferase reporter vectors were transiently transfected to N7 cells (Cellutions Biosystems, Burlington, ON, Canada) and SK-N-AS cells (American Type Culture Collection, Manassas, VA, USA). N7 cell line, derived from a mouse hypothalamic neural cell [24], is reported to express Tac2 (NKB gene in mice) and Esr1 (ERα gene) according to the manufacturer's information, and SK-N-AS cells, which originated from human neuroblastoma cells, have been used for the analysis of transcriptional regulation in human TAC3 gene [25]. N7 cells and SK-N-AS cells were maintained in DMEM (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Nichirei Biosciences, Tokyo, Japan), 1 mM sodium pyruvate (Gibco), 4 mM L-glutamine (Gibco), 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco) at 37 C in 5% CO2. Non-essential amino acids (1%) were added to the medium for SK-N-AS cells.
N7 cells and SK-N-AS cells were seeded on 24-well tissue culture plates at 2.5 × 10⁴ cells per well and 6.75 × 10⁴ cells per well, respectively, and cultured for 12 h before transfection. pGL4 vector (900 ng/well) and pRL-TK vector (100 ng/well), an internal control, were mixed with Lipofectamine 2000 Reagent (Invitrogen), and added to the cells. Twenty-four hours later, cells were treated with the medium containing vehicle (100% ethanol) or 1 or 10 nM estradiol (E2) and were incubated for an additional 24 h. Luciferase activity was determined using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer’s protocol. Transfections were performed in duplicate and repeated at least 3 times.

Statistical analysis

For analysis of the effects of E2 on luciferase activity, Student’s t-test was used. The luciferase activities of pGL-2706, pGL-1837, pGL-834, pGL-335, and pGL-197 in the non-E2-treated group were compared with that of pGL4-basic using Student’s t-test. The luciferase activities are presented as activity relative to that of the pGL4-basic vector and as means ± SEM.

Results

Identification of goat TAC3 full-length mRNA sequence

The full-length mRNA of goat TAC3 was 820 b and was deposited in the DNA Data Bank of Japan (DDBJ) database (accession no. AB796345). It consisted of 143 b of 5'-untranslated region (5'-UTR), 381 b of open reading frame (ORF), and 296 b of 3'-UTR containing a 23 b poly(A) tail (Fig. 1A). The homologies of goat TAC3 with cattle and human TAC3 were 92% and 76%, respectively. The upper end point of the 5'-UTR was estimated to be the transcription start site; this is identical to the transcription start site of cattle TAC3 and 33-b downstream of the human TAC3 transcription initiation site.

The deduced amino acid sequence of goat preproNKB, consisting of 126 residues, was compared with those of cattle and human (Fig. 1B). Goat preproNKB had 94% and 65% homology with cattle and human preproNKB, respectively. The sequence of NKB was identical among the 3 species.

Cloning of goat TAC3 5'-upstream region

We obtained goat TAC3 5'-upstream region up to 3400 b from the start codon by genome walking method (Fig. 2), and the sequence was deposited in the DDBJ database (accession no. AB797216). The putative transcription start site of goat TAC3, determined by 5'-RACE, was located in 694-b upstream of the start codon. We defined the putative transcription start point as +1; that made the upper end point of the obtained 5'-upstream region −2706 b and the translation start site +695 b. Fifteen putative EREs binding sites (half-ERE) were found in the obtained region. A putative neuron restrictive silencing element (NRSE) was found within +37 to +52 b. A GC box and CAAT box were located within −104 to −94 b and −81 to −77 b, respectively. The entire 5'-flanking sequence from −2706 to +694 b was highly conserved between goat and cattle (89%). For goat and human, the region from the start codon to −197 b had 71% homology, but the region further upstream from −198 b was dissimilar between the two species.

Promoter activity in goat TAC3 5'-upstream region

All the luciferase reporter vectors containing the goat TAC3 upstream region showed higher luciferase activity than the control vector, pGL4-basic, in N7 cells (Fig. 3B). The deletion of the goat TAC3 5'-flanking region resulted in an increase in luciferase activity and pGL-197, the shortest vector, exhibited the highest luciferase activity among the 5 vectors, showing a 55-fold increase in activity over pGL-basic. The analysis using SK-N-AS cells also demonstrated the progressive increase in luciferase activity with the deletion of the 5'-flanking region (Fig. 3C). The luciferase reporter vectors in the non-E2-treated group had significantly higher luciferase activities than pGL4-basic, except in the case of pGL-834 in N7 and pGL-2706 in SK-N-AS. The addition of E2 decreased the luciferase activity in some constructs. E2 was most effective in 10 nM E2-treated pGL-2706, the luciferase activities of which decreased to 50% (N7) and 55% (SK-N-AS) of those in the non-E2-treated group. However, the suppressive effect of E2 was not significant in either construct, including the case of pGL-2706 treated with 10 nM E2 (P=0.30 in N7 and 0.53 in SK-N-AS).

Discussion

The present study identified the sequence of full-length mRNA and the 5'-upstream region of goat TAC3. In addition, we clarified the transcriptional regulatory region of TAC3 for the first time in domestic animals using the obtained 5'-upstream region of goat TAC3.

The full-length mRNA sequence of TAC3 and putative amino acids sequence of preproNKB were highly conserved between goat and cattle (92% and 94%, respectively). In contrast, the sequences of TAC3 and preproNKB were less conserved between goat and human (76% and 65%, respectively). However, the amino acid sequence of NKB was identical between goat, cattle, and human, suggesting that the biological activity of NKB is common across these species.

The 5'-upstream sequences of goat and cattle TAC3 also showed high homology (89%), suggesting that the transcription of goat and cattle TAC3 is driven by similar mechanisms, and that the results obtained from the analysis of goat TAC3 are applicable to cattle TAC3. The fact that the putative transcription start sites of goat and cattle TAC3 were located the same distance from the start codon also supports this idea [26]. The region from the start codon to −197 bp is thought to be necessary for the basic transcription of TAC3 across species, since this region was conserved among goat, cattle, and human. In contrast, the region above −197 bp is not similar between ruminants (goat and cattle) and human, suggesting a different mechanism for TAC3 transcriptional regulation.

The luciferase assay using successive deletion of TAC3 5'-upstream revealed relatively higher luciferase activity for pGL-335 and pGL-197 than longer constructs, suggesting that the core promoter region resided in the region downstream of −197 bp. In addition, since the luciferase activity increased by the deletion of the 5'-upstream region, it is likely that some repressive elements are located in the region between −2706 and −336 bp of goat TAC3. These results were consistent with those of previous reports: Gillies et al. evaluated the promoter activity of the human TAC3 5'-upstream region by luciferase assay using SK-N-AS cells and showed that the −289 to +181 bp region had higher activity than the −757 to +181 bp region.
Fig. 1. Identification of goat TAC3 gene. (A) Full-length mRNA and deduced amino acid sequences of goat TAC3 gene. The deduced amino acids are shown under the nucleotide sequence in single-letter notation. Asterisk (*) indicates stop codon. Numbers indicate nucleotide positions. (B) Alignment of amino acid sequences of goat, cattle, and human preproNKB. Identical (*), conserved (:), and semi-conserved (.) amino acid residues are marked. The amino acid sequences of NKB are highlighted with grey shading.
Fig. 2. Sequence of the 5'-flanking region from –2706 to +166 b of goat Tac3 gene. The numbering of nucleotide residues on the left is relative to a putative transcription start site designated as +1 (upstream, –; downstream, +). The start codon (ATG) is found 694 b-downstream of the putative transcription start site. The estimated binding sites of estrogen receptor α (half-ERE), GC boxes, a CAAT box, and a putative neuron restrictive silencing element (NRSE) binding site are indicated.
been known to recognize a 21-bp consensus sequence [28, 29], binding to NRSE located within +50 to +70 b [25, 27]. NRSF has silencing factor (NRSF) activates human TAC3 transcription by similar manner. Gillies et al. demonstrated that neuron restrictive TAC3 genes is regulated in a similar manner to the –196 to +166 bp of goat TAC3. The –289 to +181 bp region of human TAC3 is equivalent to the –197 to +166 bp of goat TAC3, indicating that the core promoter of the goat and human TAC3 genes is regulated in a similar manner. Gillies et al. demonstrated that neuron restrictive silencing factor (NRSF) activates human TAC3 transcription by binding to NRSE located within +50 to +70 b [25, 27]. NRSF has been known to recognize a 21-bp consensus sequence [28, 29], NRSE, and the core sequence is included in this consensus sequence [29]. In the goat TAC3 5'-upstream region, an NRSE-like sequence existed 10-b downstream of human TAC3; however, one base of the goat TAC3 NRSE-like sequence was different from the NRSE core sequence. Further research is needed to know whether NRSF acts as a transcriptional activator of goat TAC3 similarly to human TAC3.

The complex of sex steroid and its receptor is known to act as a transcription factor. Generally, ERα regulates the transcription of genes in collaboration with another transcription factor, such as Sp-1 or AP-1 [30, 31]. A previous study in ovariectomized mice reported that E2 treatment decreased the number of Tac2 mRNA-expressing cells in the ARC by 53% compared with the control [15]. Ovariectomized sheep treated with E2 also exhibited a significant decrease in TAC3 mRNA-expressing cells in the hypothalamus [32]. Additionally, E2 treatment decreased the Tac2 expression level in the ARC in wild-type mice but not in ERα knockout mice [33]. These previous results indicate that estrogen negatively regulates TAC3 transcription via ERα. Analysis of the predicted transcription factor binding sites found a number of binding sites for ERα (half-ERE), Sp-1, and AP-1 in the 5’-upstream region of goat TAC3. This may indicate the involvement of estrogen as a suppressive factor of TAC3; therefore, we examined the effect of E2 on the luciferase activity of each construct. The mean luciferase activities of pGL-2706, pGL-1837, and pGL-834 were lower in the 1 nM E2-treated group, which may be responsible for a number of the ERα binding sites included in those vectors: The regions from –2706 to –1838 bp, –1837 to –835 bp, and –834 to –336 bp contained 7, 3, and 4 putative ERα binding sites, respectively (Fig. 3A). The luciferase activity in pGL-197 also had a tendency to be suppressed by E2. We could not find an ERα binding site within the region from –197 to +166 bp, but this region contained several Sp-1 binding sites; therefore, it is possible that ERα acts indirectly on the region by binding and collaborating with Sp-1. Treatment with 10 nM E2 suppressed the luciferase activity of pGL-2706 by approximately half, which was the highest suppression ratio, indicating that multiple ERα binding sites located in the region from –2706 to +166 bp act as transcriptional suppressive elements. However, all the above-mentioned suppressive effects of E2 were not statistically significant. Estrogen is likely to have a certain effect on TAC3 suppression but may not be a major factor that regulates TAC3 transcription in goats. Thus, the effect of estrogen on goat TAC3 transcription was not fully established in the current experiment, which was different from the results in previous reports using in vivo animal studies. The present results of our promoter assay suggested that estrogen does not directly act on TAC3 transcription, although it may indirectly influence the decrease in goat TAC3 expression through an unknown mechanism.

It may also be possible that the putative ERα binding sites in the –2706 to +166 bp region are not the actual binding sites of ERα but the sequences outside the –2706 to +166 bp region are responsible for suppression of TAC3 transcription by estrogen. One possible limiting factor of our study is that the N7 and SK-N-AS cells used in the luciferase assays may have been insufficient to fully detect the effects of estrogen on TAC3 transcription. For example, a factor(s) that works with ERα may not have been enough in these cell lines to cause a significant reduction in luciferase activity by E2 treatment.

In conclusion, goat TAC3, encoding NKB, is highly homologous.
to cattle TAC3 in both the mRNA and 5'-upstream region sequences. The present study demonstrated the prerequisite regions for the gene expression of goat TAC3: the downstream region of –197 bp includes the core promoter of goat TAC3, and the region from –2706 to –336 bp includes transcriptional suppressor. The results of this study also suggested that estrogen does not impart a direct effect on goat TAC3 transcription. Further studies on the identification of the major regulator(s) of TAC3 transcription are thus warranted.

Acknowledgments

We thank Ms K Yamazaki and Mr Y Kono for their careful animal care and technical assistance. This work was supported in part by a Grant-in-Aid for Research Activity Start-up Grant to FM (23880012) and Grants-in-Aid for Scientific Research to SO in part by a Grant-in-Aid for Research Activity Start-up Grant to JK.

References

1. Belchetz PE, Plant TM, Nakai Y, Koshg EJ, Ksobil E. Hypophysial responses to continuous and intermittent delivery of GnRH of hypothalamic gonadotropin-releasing hormone. Science 1978;202:631–633. [Medline]
2. Dierschke DJ, Bhattacharya AN, Atkinson LE, Ksobil E. Circhoral oscillations of plasma LH levels in the ovaricized rhesus monkey. Endocrinology 1970;87:850–853. [Medline]
3. Ksobil E, Plant TM, Wildt L, Belchetz PE, Marshall G. Control of the rhesus monkey menstrual cycle: permissive role of hypothalamic gonadotropin-releasing hormone. Science 1980;207:1371–1373. [Medline]
4. Radovich S, Levine JE, Wolfe A. Estrogenic regulation of the GnRH neuron. Front Endocrinet (Lausanne) 2012;3:52. [Medline]
5. Herbison AE. Multimodal influence of estrogen upon gonadotropin-releasing hormone neurons. Endocr Rev 1998;19:302–330. [Medline]
6. Oakley AE, Clifton DK, Steiner RA. Kisspeptin signaling in the brain. Endocr Rev 2009;30:713–743. [Medline]
7. Rance NE. Menopause and the human hypothalamus: evidence for the role of kisspeptin/neurokinin B neurons in the regulation of estrogen negative feedback. Peptides 2009;30:111–122. [Medline]
8. Kauffman AS. Sexual differentiation and the Kiss1 system: hormonal and developmental considerations. Peptides 2009;30:83–93. [Medline]
9. Clarkson J, Boon WC, Simpson ER, Herbison AE. Postnatal development of an estradiol-kisspeptin positive feedback mechanism implicated in puberty onset. Endocrinology 2009;150:3214–3220. [Medline]
10. Adachi S, Yamada S, Takatsu Y, Matsui H, Kinoshita M, Takase K, Sugijura H, Obtaki T, Matsushita H, Uenoyma Y, Tsukamura H, Inoue K, Maeda KI. Involvement of anteroventral periventricular metastin/kisspeptin neurons in estrogen positive feedback action on luteinizing hormone release in female rats. J Reprod Dev 2007;53:367–378. [Medline]
11. Franceschini I, Lomet D, Cateau M, Delos G, Tillet Y, Caraty A. Kisspeptin immunoreactive cells of the ovine preoptic area and arcuate co-express estrogen receptor alpha. Neurosci Lett 2006;401:225–230. [Medline]
12. Tsunikawa J, Homma T, Tajima S, Shihata T, Inamoto Y, Takase K, Inoue N, Okhura S, Uenoyma Y, Maeda KI, Tsukamura H. Molecular characterization and estrogen regulation of hypothalamic KISS1 gene in the pig. Biol Reprod 2010;82:313–319. [Medline]
13. Okhura S, Uenoyma Y, Yamada S, Homma T, Takase K, Inoue N, Maeda KI, Tsukamura H. Physiological role of metastin/kisspeptin in regulating gonadotropin-releasing hormone (GnRH) secretion in female rats. Peptides 2009;30:49–56. [Medline]
14. Navarro VM, Castellano JM, McConkey SM, Pineda R, Ruiz-Pino F, Pinilla L, Cliffon DK, Tena-Sempere M, Steiner RA. Interactions between kisspeptin and neurokinin B in the control of GnRH secretion in the female rat. Am J Physiol Endocrinol Metab 2011;300:E202–E210. [Medline]
15. Navarro VM, Gottsch ML, Chavkin C, Okamura H, Clifton DK, Steiner RA. Regulation of gonadotropin-releasing hormone secretion by kisspeptin/dynorphin/neurokinin B neurons in the arcuate nucleus of the mouse. J Neurosci 2009;29:11859–11866. [Medline]
16. Goodman RL, Lehman MN, Smith JT, Coolen LM, De Oliveira CV, Jafarzadehrazai MR, Pereira A, Ipahal J, Caraty A, Cloot P, Clarke LJ. Kisspeptin neurons in the arcuate nucleus of the ewe express both dynorphin A and neurokinin B. Endocrinology 2007;148:5752–5760. [Medline]
17. Ramaswamy S, Seminara SB, Ali B, Cloot P, Amin NA, Plant TM. Neurokinin B stimulates GnRH release in the male Monkey (Macaca mulatta) and is colocalized with kisspeptin in the arcuate nucleus. Endocrinology 2010;151:4944–4953. [Medline]
18. Pokbayashi Y, Nakada T, Murata K, Okhura S, Mogi K, Navarro VM, Clifton DK, Mori Y, Tsukamura H, Maeda KI, Steiner RA, Okamura H. Neurokinin B and dynorphin A in kisspeptin neurons of the arcuate nucleus participate in generation of periodic oscillation of neural activity driving pulsatile gonadotropin-releasing hormone secretion in the goat. J Neurosci 2010;30:3124–3132. [Medline]
19. Lehman MN, Coolen LM, Goodman RL. Minireview: kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: a central node in the control of gonadotropin-releasing hormone secretion. Endocrinology 2010;151:3479–3489. [Medline]
20. Rance NE, Krajewski SJ, Smith MA, Cholian M, Dacks PA. Neurokinin B and the hypothalamic regulation of reproduction. Brain Res 2010;1364:116–128. [Medline]
21. Topaloglu AK, Reimann F, Guelu M, Yalin AS, Kotan LD, Porter KM, Serin A, Mungan NO, Cook JR, Ozbek MN, Imamoglu S, Akalin NS, Yuskeli B, ORahilly S, Semple RK. TAC3 and TAC3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. Nat Genet 2009;41:354–358. [Medline]
22. Young J, Boullang J, Francou B, Rafin-Sanson ML, Galilez S, Jeanpierre M, Gryberg M, Kamenicky P, Chanson P, Brailly-Tabard S, Guisouh-Valent M. TAC3 and TAC3 defects cause hypothalamic congenital hypogonadotropic hypogonadism in humans. J Clin Endocrinol Metab 2010;95:2287–2295. [Medline]
23. Noriike K, Matsuoka T, Ohawa T, Shimosura K, Sanbushio A, Uenoyma Y, Maeda KI, Tsukamura H. Involvement of neurokinin receptors in the control of pulsatile luteinizing hormone secretion in mice. J Reprod Dev 2011;57:409–415. [Medline]
24. Belsham DD, Cal F, Cui H, Smukler SR, Salapatek AMF, Shkreta L. Generation of a phenotypic array of hypothalamic neuronal cell models to study complex neuroendocrine disorders. Endocrinology 2004;145:393–408. [Medline]
25. Gillies S, Haddley K, Vasiliss S, Bubb VJ, Quin JP. The human neurokinin B gene, TAC3, and its promoter are regulated by Neuron Restrictive Silencing Factor (NRSF) transcription factor family. Neuropeptides 2009;43:333–346. [Medline]
26. Kotani H, Hoshimaru M, Nawa H, Nakashima S. Structure and gene organization of bovine neurokinin K precursor. Proc Natl Acad Sci USA 1986;83:7074–7078. [Medline]
27. Spencer EM, Chandler KE, Haddley K, Howard MR, Hughes D, Belyaev ND, Coulson JM, Stewart JP, Buckley NJ, Kipar A, Walker MC, Quinn JP. Regulation and role of REST and REST4 variants in modulation of gene expression in vivo and in vitro in epilepsy models. Neurobiol Disease 2006;24:41–52. [Medline]
28. Otto SJ, McCorkle SR, Hover J, Conaco C, Han J-D, Impey S, Yoshum GS, Duan JJ, Goodman RH, Mandel G. A new binding motif for the transcriptional repressor REST uncoverts large gene networks devoted to neuronal functions. J Neurosci 2007;27:6729–6739. [Medline]
29. Wu J, Xie X. Comparative sequence analysis reveals an intricate network among REST, CREB and mRNA in mediating neuronal gene expression. Genome Biol 2006;7:R58. [Medline]
30. Safe S, Kim K. Nuclear receptor-mediated transactivation through interaction with Sp proteins. Prog Nucleic Acid Res Mol Biol 2004;77:1–36. [Medline]
31. Gruber CJ, Gruber DM, Gruber JM, Wieser F, Huber J, C. Anatomy of the estrogen response element. Trends Endocrinol Metab 2004;15:73–78. [Medline]
32. Pilton D, Caraty A, Fabre-Nys C, Bruneau G. Short-term effect of oestradiol on neurokinin B mRNA expression in the infundibular nucleus of ewes. J Neuroendocrinol 2003;15:749–753. [Medline]
33. Dellovade TL, Merchenthaler I. Estrogen regulation of neurokinin B gene expression in the mouse arcuate nucleus is mediated by estrogen receptor alpha. Endocrinology 2004;145:736–742. [Medline]