Cloning and Expression of β1-Adrenergic Receptor Genes in Adipose Tissues from Korean Native Cattle (Hanwoo)

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ABSTRACT : Bovine β1-adrenergic receptor (AR) cDNA was cloned using degenerative primers. Bovine β1-AR coded for 467 amino acids and the comparison of the deduced amino acid sequence with that of sheep showed 93.4% identity. Northern blot analysis indicated that transcript size for the bovine β1-AR was 3.6 kb in the adipose tissue. The expression level of three β-ARs (1, 2, and 3) in bovine abdominal, subcutaneous, and perirenal adipose tissues were examined using reverse transcription-polymerase chain reaction (RT-PCR), and the levels of β1- and β3-AR mRNA were found to be lower in the subcutaneous adipose tissue than in the abdominal and perirenal adipose tissues. These results suggest that the expression of β-ARs mRNA are differentially regulated among the adipose tissues. *(Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 1 : 13-16)*

Key Words : β1-Adrenergic Receptor cDNA, Cloning, Expression, Adipose Tissue

INTRODUCTION

Adrenergic receptors (AR) consist of alpha and beta subtypes and members of the family of guanine-nucleotide-binding and adenylyl-cyclase-coupled proteins. These receptors are presumed to contain seven transmembrane helices. Three different β-ARs (β1-, β2-, and β3-AR) have been identified in various tissues and species including human, mouse, rat, guinea pig and hamster (Nahmias et al., 1991). Using these clones, the expression of all three β-AR subtypes and different regulatory patterns for each subtypes have been elucidated in rat and porcine adipocytes (Portillo et al., 1996; Mcneel et al., 1995).

Also, the catecholamines, epinephrine and norepinephrine are effector molecules for the receptors (Strosberg, 1993) and β1- and β2-AR present a higher affinity for catecholamines than β3-AR (Galitzky et al., 1995). Stimulation of β-ARs suppresses lipid synthesis and induces lipolysis (Bousquet-Melou et al., 1995).

In this study, we cloned bovine β1-AR cDNA from genomic DNA of Korean cattle (Hanwoo). Using reverse transcription-polymerase chain reaction (RT-PCR), we examined the expression patterns of β-AR subtypes in the abdominal, subcutaneous, and perirenal adipose tissue of Hanwoo Steers.

MATERIALS AND METHODS

Tissue samples and isolation of genomic DNA and total RNA

Adipose tissues were prepared from abdominal, subcutaneous, and perirenal adipose tissues of 48 month old Korean cattle (Hanwoo). The adipose tissues were incubated in the DNA extraction buffer containing 1% sodium dodecyl sulfate and proteinase K. After incubation for 12 h at 37℃, samples were extracted with phenol/chloroform and then precipitated with ethanol. DNA was dissolved in TE buffer and analyzed on a 1% agarose gel. Total RNA was isolated from adipose tissues by the guanidine isothiocynate method (Chomczyski and Sacchi, 1987) and analyzed on a 1% formaldehyde agarose gels.

Genomic PCR for β1-AR cloning

It has been known that β1-AR genes of the several species (rat, pig, sheep, and human) have no intron. Therefore we used genomic DNA for the PCR template. Genomic PCR was carried out in 50 μl reaction mixture containing 150 ng genomic DNA, 1× PCR buffer, 2 mM $MgCl_2$, 200 μM each dNTP, 5% DMSO, 1.25 U Taq DNA polymerase, and 0.5 pmol 5' degenerative and 0.2 pmol 3' gene-specific primers or 0.2 pmol 5' gene-specific and 0.5 pmol 3' degenerative primers. Degenerative primers were derived from highly conserved nucleotide sequences of rat (Shimonura and Terada, 1990), pig (Cao et al., 1998), sheep (Padbury et al., 1995), and human (Frielle et al., 1987). Sense primer (5'-TYCCCRRSCR CGGCCCARYYYYG-3') and anti-sense (5'-YCTACAC CTTGGAYTCSGAGSMG-3') primers were designed to contain the 5-noncoding region and the region containing stop codon (M: A or C, R: A or G, S: G or C, and Y: C or T), respectively. Gene specific primers were derived from the region showing 100% identity among rat, pig, and human (sense: 5'- TGCA GACGCTCACCAACCTCTTC-3', anti-sense: 5'-GGCT GCGGCAGTAGATGATGGGG-3'). At first step, PCR

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reaction mixture was subjected to 5 cycles of denaturation at 95℃ for 20 sec, annealing at 66-72℃ for 10 sec, and extension at 72℃ for 90 sec. The annealing temperature of each cycle was decreased by 1.5℃. The second step was subjected to 30 cycles of denaturation at 95℃ for 20 sec, annealing at 65℃ for 5 sec, and extension at 72℃ for 90 sec.

Northern blot anlaysis and sequencing

The mRNA was isolated with mRNA isolation system III (Promega) as described in the manual. Northern blot analysis was performed with 5 μg mRNA. Sequencing reaction were performed with ABI™ BigDye™ Terminator (Perkin-Elmer, USA) as described by manual. The reaction mixture was electrophoresed on 6% polyacrylamide gel using ABI™ Prism 377 DNA sequencer (Perkin-Elmer).

Reverse transcription-polymerase chain reaction (RT-PCR)

cDNA was synthesized in 20 μl reaction mixture using 5 μg total RNA and reverse transcriptase. PCR amplification was performed in 50 μl reaction mixture using 2 μl of the RT products, 1×PCR buffer, 2 mM $MgCl_2$, 200 μM each dNTP, 5% DMSO, 1.25 U Taq DNA polymerase, and each β-AR gene specific primers (β1-AR sense 5'-CCAGAAGCAGGTGAAGA AGATCG-3', anti-sense 5'-CAGTTGAAGAAGACGAA GAGGC-3'; β2-AR sense 5'-TGGTCATGGTCTTCGT CTACTCC-3', anti-sense 5'-CAGTCCTGTCATTGCTG TTGCTG-3'; β3-AR sense 5'-TCTATCTTCCGCTCCT GGTGATG-3', anti-sense 5'-GGTTGAAGGCAGAGTT GGCATAG-3'). Primers specific to bovine β-actin gene (sense 5'-GGCACCACACCTTCTACAAGGAG-3', anti-sense 5'-GCTTCTCCTTGATGTCACAGACG-3') were also added to the PCR reaction mixture to serve as an internal control. PCR reaction mixtures were subjected to 46, 33, 40, and 37 cycles of denaturation at 95℃ for 20 sec, annealing at 61℃ for 30 sec, and extension at 72℃ for 60 sec for β1, β2, β3, and β-actin, respectively. The PCR products were analyzed on a 1.5% agarose gel.

RESULTS AND DISCUSSION

It has been known that β1-AR genes of the

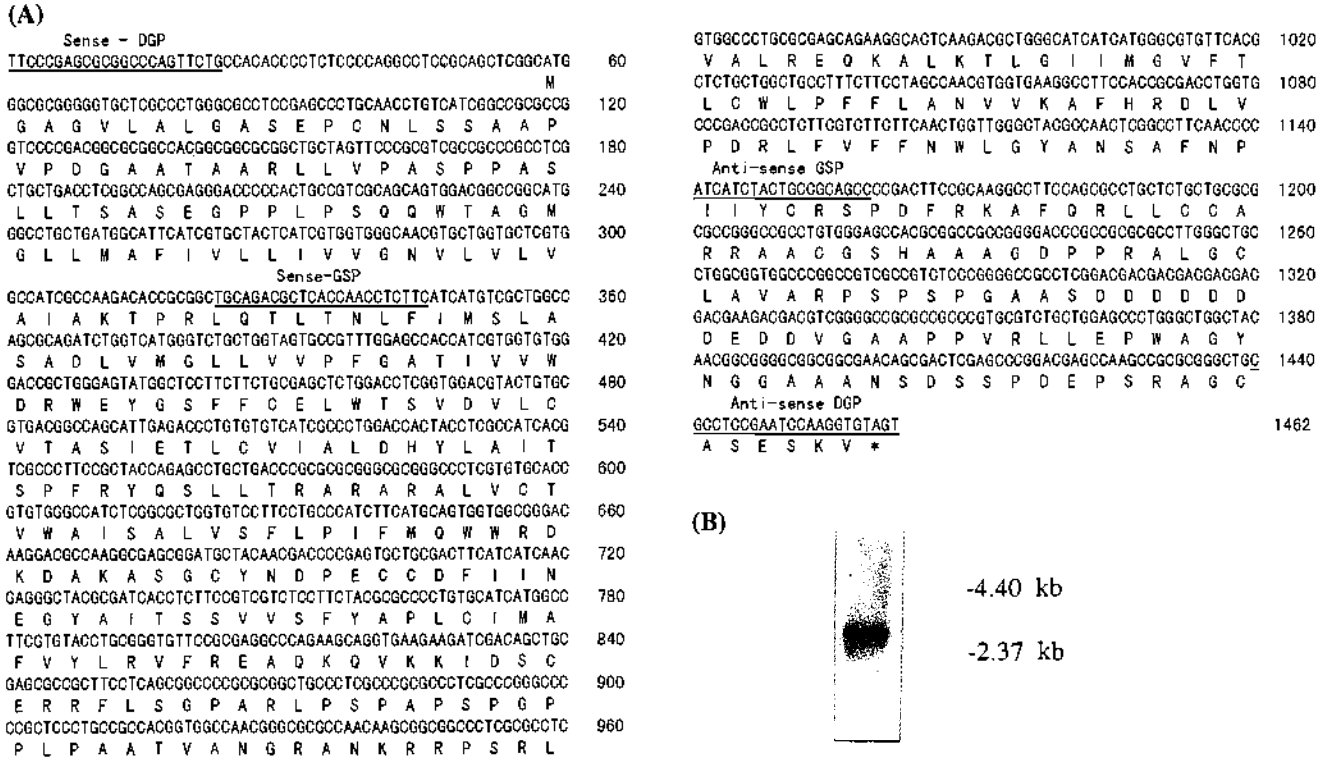

Figure 1. Nucleotide and deduced amino acid sequences of bovine β1-AR gene and Northern blot analysis in adipose tissue. (A) The bovine β1-AR clone contained 1,462 bp, and amino acid sequences are given in single letter code underneath the nucleotide sequences. The box shows degenerative (DGP) and gene specific (GSP) primers used for PCR. The nucleotide sequence data reported in this paper are in the EMBL, GenBank, and DDBJ nucleotide sequence databases with the accession number AF188187. (B) Five μg mRNA isolated from bovine adipose tissue was loaded, and Northern blot analysis was performed using ^{32}P-labeled bovine β1-AR probe.

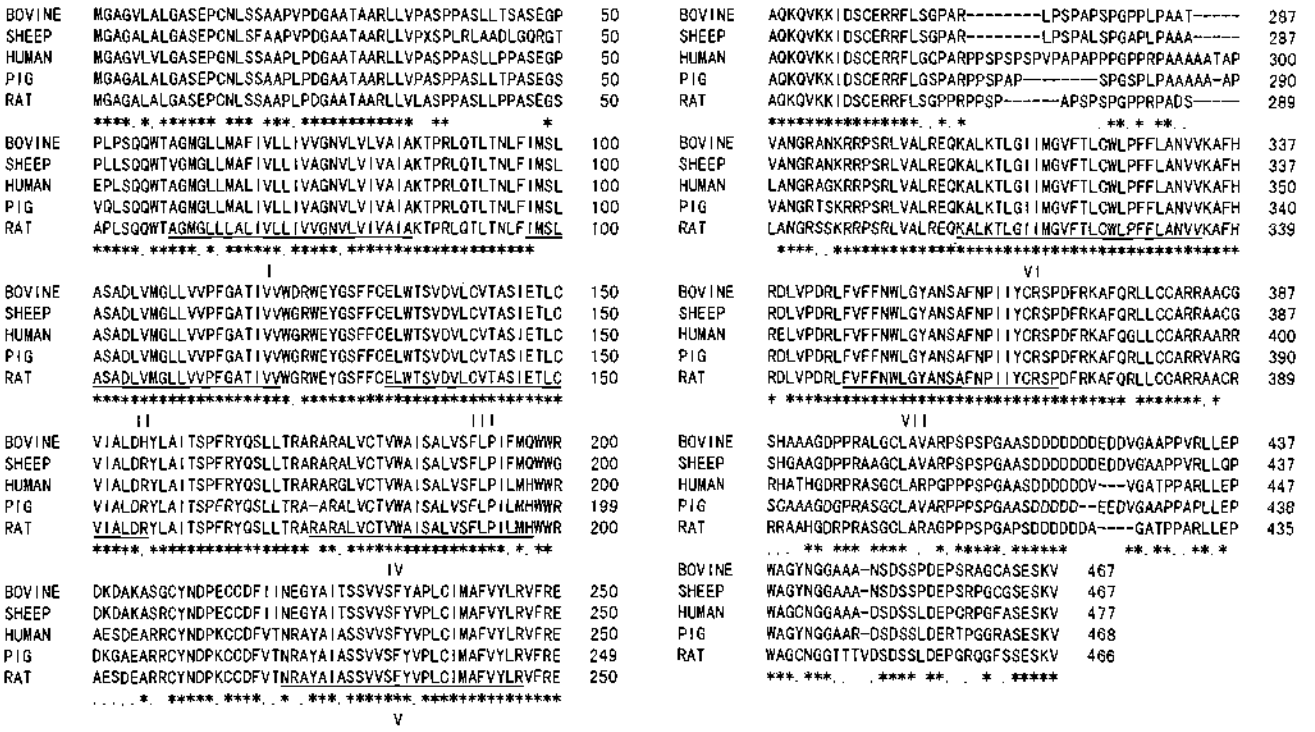

Figure 2. Comparison of deduced amino acid sequence of β1-AR. The multiple alignment was done on five protein sequences (bovine, rat, pig, sheep, and human β1-AR). The underlined portions denote transmembrane region. Star (*) shows that a position in the alignment is perfectly conserved and dot (.) shows that a position is well conserved.

several species have no intron and we speculate that its expression would be low. Therefore, we cloned the bovine β1-AR from genomic DNA using sense degenerative and anti-sense gene-specific primers or sense gene-specific and anti-sense degenerative primers. After genomic PCR and sequencing (n=3), we obtained approximately 1.1 kb each clones. The combined sequence of the two clones produced bovine β1-AR gene containing full-coding region which was 1,462 nucleotides in length and coded for 467 amino acids (figure 1A). β2- and β3-AR coding sequences were also cloned in Hanwoo. The coding region sequences of Hanwoo were identical to those of the published bovine cDNA sequences (Pietri-Rouxel et al., 1995) (data not shown). In order to measure the transcript size of the bovine β1-AR in adipose tissue, we performed northern blot analysis with purified mRNA. As shown in figure 1B, the size of the β1-AR transcript was approximately 3.6 kb.

Comparison of the deduced amino acid sequence of bovine β1-AR to those of other β1-AR showed low similarities to those from monogastric animals (60.1, 66,6, and 65.3% identities with rat, pig, and human, respectively) and high similarities to those from ruminants (93.4% identity with sheep). The sequence of a putative transmembrane regions of β1-AR amino acid showed a very high identity (94-97%). In particular, transmembrane regions, II, VI, and VII in monogastric animal (rat, pig, and human) were identical with those of the ruminant (sheep and bovine) (figure 2).

To examine the expression of β-AR subtypes, we carried out RT-PCR with abdominal, subcutaneous, and perirenal adipose tissues of Hanwoo steers. The estimated β-ARs mRNA levels were corrected with these corresponding to β-actin. The expression levels of β1- and β3-AR mRNAs were seven to eight and two times lower, respectively, in the subcutaneous adipose tissue than in the abdominal and perirenal adipose tissues. The expression levels of β2-AR mRNA were slightly higher in the subcutaneous and perirenal adipose tissues than in the abdominal adipose tissue. (figure 3). These results are similar with the expression patterns of β1 in porcine adipose tissues; indeed, the expression of β1-AR transcripts is higher in perirenal depot than in subcutaneous (Mcneel and Mersmann, 1995).

Catecholamines and glucocorticoids can alter transcription of β-AR gene (Collins et al., 1988). Norepinephrine stimulates lipolysis through β1-AR at low plasma levels and β3-AR at high plasma levels. β2-AR play only a minor role (Atgie et al., 1997). Therefore, the differences in expression of each β-ARs mRNA in different adipose tissues may be due

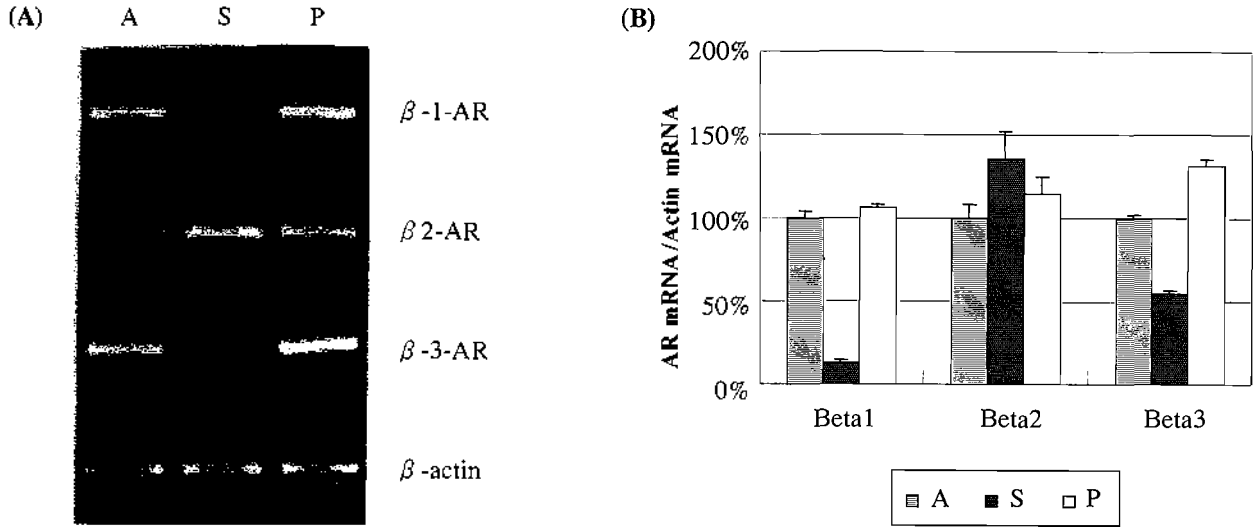

Figure 3. Expression of β-AR subtypes in bovine adipose tissues. (A) Total RNA was isolated from abdominal (A), subcutaneous (S), and perirenal (P) adipose tissues and used for RT-PCR. Two μl of cDNA from adipose tissues were amplified with each β-AR subtypes and β-actin primers. (B) Relative levels of β-AR mRNAs were normalized with corresponding β-actin levels. mRNA levels at abdominal adipose tissue was expressed as 100%, and bar indicates standard deviation of three-independent experiments.

to differential regulation of hormones or other agents. Also, our results suggest that differenes in each β-ARs mRNA expression in different adipose tissues could contribute to the differences in lipid metabolism among the adipose tissues.

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