Available online at www.sciencedirect.com
ScienceDirect
Domestic Animal Endocrinology 33 (2007) 19–31
DOMESTIC ANIMAL ENDOCRINOLOGY
www.journals.elsevierhealth.com/periodicals/dae

Molecular cloning and tissue expression of chicken AdipoR1 and AdipoR2 complementary deoxyribonucleic acids☆

Ramesh Ramachandran*, Olga M. Ocón-Grove, Shana L. Metzger

Department of Poultry Science, The Pennsylvania State University, University Park, PA 16802, USA

Received 24 October 2005; received in revised form 4 April 2006; accepted 6 April 2006

Abstract

AdipoR1 and AdipoR2 belong to a novel class of transmembrane receptors that mediate the effects of adiponectin. We have cloned the chicken AdipoR1 and AdipoR2 complementary deoxyribonucleic acids (cDNA) and determined their expression in various tissues. We also investigated the effect of feed deprivation on the expression of AdipoR1 or AdipoR2 mRNA in the chicken diencephalon, liver, anterior pituitary gland, and adipose tissue. The chicken AdipoR1 and AdipoR2 cDNA sequences were 76–83% identical to the respective mammalian sequences. A hydrophobicity analysis of the deduced amino acid sequences of chicken AdipoR1/AdipoR2 revealed seven distinct hydrophobic regions representing seven transmembrane domains. By RT-PCR, we detected AdipoR1 and AdipoR2 mRNA in adipose tissue, liver, anterior pituitary gland, diencephalon, skeletal muscle, kidney, spleen, ovary, and blood. AdipoR1 or AdipoR2 mRNA expression in various tissues was quantified by real-time quantitative PCR, and AdipoR1 mRNA expression was the highest in skeletal muscle, adipose tissue and diencephalon, followed by kidney, ovary, liver, anterior pituitary gland, and spleen. AdipoR2 mRNA expression was the highest in adipose tissue followed by skeletal muscle, liver, ovary, diencephalon, anterior pituitary gland, kidney, and spleen. We also found that a 48 h feed deprivation significantly decreased AdipoR1 mRNA quantity in the chicken pituitary gland, while AdipoR2 mRNA quantity was significantly increased in adipose tissue ($P < 0.05$). We conclude that the Adi-

☆ The chicken AdipoR1 and AdipoR2 cDNA sequences have been submitted to GenBank Database under Accession no. DQ072275 (AdipoR1), DQ072276 (AdipoR2).

* Corresponding author. Tel.: +1 814 865 5202; fax: +1 814 865 5691.
E-mail address: RameshR@psu.edu (R. Ramachandran).

doi:10.1016/j.domaniend.2006.04.004

poR1 and AdipoR2 genes are ubiquitously expressed in chicken tissues and that their expression is altered by feed deprivation in the anterior pituitary gland and adipose tissue.

Keywords: Adiponectin; Diencephalon; Pituitary gland; Liver; Kidney; Energy homeostasis; Adipose tissue

1. Introduction

Adiponectin (also called Acrp30, apM1, GBP28, or adipoQ) is a 30 kDa adipokine hormone secreted primarily from adipose tissue in several mammalian species [1–4]. It is a 244-amino acid protein belonging to a family of proteins that contain sequences homologous to the C1q globular domain. Adiponectin consists of a N-terminal collagenous domain and a C-terminal globular domain [5,6]. The globular domain is sufficient to produce various biological effects of adiponectin [7]. Adiponectin stimulates glucose utilization and fatty acid oxidation by phosphorylating and activating 5′-AMP-activated protein kinase (AMPK) [8]. Activation of AMPK results in phosphorylation of a variety of intracellular proteins and an increase in ATP generation [9]. In addition, adiponectin increases fatty acid oxidation through inhibition of acetyl-Coenzyme A (Co-A) carboxylase and activation of malonyl-CoA decarboxylase resulting in reduced malonyl-CoA content [10]. A decrease in malonyl-CoA concentration increases the transport of long chain fatty acyl-CoA molecules into the mitochondria where they are oxidized [11].

Recently, two complementary deoxyribonucleic acids (cDNA) encoding two distinct adiponectin receptors have been cloned and designated as AdipoR1 and AdipoR2 [12]. The deduced amino acid sequence of both AdipoR1 and AdipoR2 have been predicted to have seven transmembrane domains but they differ from traditional G-protein coupled receptors (GPCR). Unlike GPCR, the N-terminal end of AdipoR1 and AdipoR2 is localized intracellularly while their C-terminal ends reside in the extracellular domain [12]. AdipoR1 has been reported to have greater binding affinity to the globular domain of adiponectin while AdipoR2 binds to both the globular and full length adiponectin with intermediate affinity [12]. AdipoR1 is expressed ubiquitously, with the most abundant expression occurring in the mouse skeletal muscle while AdipoR2 is predominantly expressed in the liver [12]. Both the AdipoR1 and AdipoR2 genes are expressed in human monocytes and macrophages [13] and also in a primary human osteoblast cell line [14]. In addition, AdipoR1 and AdipoR2 genes are expressed in rat and human pancreatic beta cells at levels similar to those expressed in the liver, but greater than in muscle [15]. Both AdipoR1 and AdipoR2 have been found to mediate the metabolic effects of adiponectin.

AdipoR1 and AdipoR2 have been found to be highly conserved molecules in several mammalian species [12]. However, adiponectin receptors in avian species have not been studied in the past. We earlier characterized adiponectin cDNA in the chicken and its unique expression in several tissues including adipose tissue [16]. Cloning of the chicken adiponectin receptor gene sequences will help to advance our knowledge on the metabolic effects of adiponectin in this species that has physiologically higher levels of blood glucose compared to mammals [17]. Unlike rodents and other non-primate mammalian species, fatty acid synthesis occurs primarily in the liver of the chicken (as in the human) rather

than adipose tissue [18]. Therefore, cloning adiponectin receptors in the chicken will lead to a better understanding of the role of adiponectin signaling in lipogenesis. Here, we describe cloning of chicken AdipoR1 and AdipoR2 cDNA from adipose tissue and provide novel evidence that both AdipoR1 and AdipoR2 are expressed in multiple tissues including diencephalon and anterior pituitary gland. We also provide evidence that expression of the genes encoding AdipoR1 and AdipoR2 in anterior pituitary gland and adipose tissue is significantly altered by feed deprivation.

2. Materials and methods

2.1. Animals

Adult female chickens (Hyline W36 strain) were housed in cages and provided water and feed ad libitum unless otherwise indicated. All animal procedures were carried out in accordance with the Institutional Animal Care and Use Committee approved protocol. Chickens were fasted for 48 h to determine the effect of fasting on AdipoR1 and AdipoR2 gene expression in various tissues. Water was available to the chickens at all times during fasting.

2.2. Cloning of cDNAs encoding chicken AdipoR1/AdipoR2

AdipoR1 and AdipoR2 cDNAs were cloned from total RNA extracted from chicken adipose tissue. Total RNA was extracted from chicken adipose tissue using Trizol (Invitrogen, Carlsbad, CA). Following DNAse-I (Qiagen, Valencia, CA) treatment, first strand cDNA was synthesized by reverse transcribing 1 μg of total RNA using $d(T)_{30}$A/G/CA/G/C/T primer and 2U of Powerscript reverse transcriptase (Clontech, Palo Alto, CA). Forward and reverse primers were developed from a highly conserved region of mammalian (human, mouse, and swine) AdipoR1 or AdipoR2 cDNA (GenBank Accession nos.: NM_015999; NM_024551) to amplify a 368 and 389 bp partial cDNA encoding chicken AdipoR1 and AdipoR2, respectively. The polymerase chain reaction (PCR) consisted of 300 nM of the forward and reverse primers (AdipoR1: forward-CTGCCTCAGCTTCTCCTGGCT; reverse-AGRAAGAACCAGCCCATCT; AdipoR2: forward-GAAGGGTTTATGGGCATGTC; reverse-GAAGAGCCATGAAAAGGAAA), 300 nM of dNTP mixture (Invitrogen) and 2.5U Deep $Vent_R$ polymerase (New England BioLabs, Beverly, MA). Using a DNA Engine PTC-200 thermal cycler (MJ Research, Reno, NV), PCR was conducted with the following thermocycle: 94 °C for 1 min, 30 cycles of 94 °C for 5 s, 58 °C for 10 s and 62 °C for 30 s with a final extension of 62 °C for 10 min. The PCR products were gel purified and subcloned into pGEM-T Easy vector (Promega, Austin, TX) and both sense and antisense strands were sequenced (Davis Sequencing, Davis, CA) using Sp6 and T7 primers. Chicken AdipoR1 or AdipoR2-gene specific primers were designed from the above partial cDNA sequences for 5′/3′ rapid amplification of cDNA end (RACE) reactions using a SMART RACE kit (Clontech). The 5′ and 3′ ends of the chicken AdipoR1 and AdipoR2 cDNAs were then amplified following manufacturer's instructions and subcloned into pGEM-T Easy (Promega) and sequenced.

2.3. RT-PCR

Total RNA was extracted from chicken ($n = 4$; sexually mature 35 weeks old leghorn females) adipose tissue, liver, blood, anterior pituitary gland, diencephalon, skeletal muscle (breast muscle), spleen, ovary and kidney using Trizol (Invitrogen) and/or by using RNeasy kit (Qiagen). Following DNAse-I (Qiagen) treatment, first strand cDNA was synthesized by reverse transcribing 1 μg of total RNA using $d(T)_{30}$A/G/C A/G/C/T primer and 2U Powerscript reverse transcriptase (Clontech). Approximately, 100 ng of single stranded cDNA was used as template to amplify a 350 and 345 bp product of AdipoR1 and AdipoR2, respectively, using the following primers (AdipoR1: forward-gaatacacaccgagacgggcaacatct; reverse-gcccaagacgcagacaatggagaggta; AdipoR2: forward-gagactggcaacatctggacgcatcttc; reverse-tgcgatgcccaggacacaaatcacaat). A touch-down PCR was performed using the following program: 94 °C for 1 min, 30 cycles of 94 °C for 5 s and 72–68 °C for 3 min. Annealing and primer extension were done at 72, 70, and 68 °C during 1–5, 6–10, and 11–30 cycles, respectively. The PCR products were subjected to agarose gel electrophoresis and ethidium bromide staining for visualization. For negative control, reverse transcription reaction using 1 μg total RNA from each tissue with no reverse transcriptase (−RT control) or water was used as template in place of single stranded cDNA (+RT). The RT-PCR product from each tissue was sequenced to confirm authenticity.

2.4. AdipoR1 mRNA/AdipoR2 mRNA quantitation in various tissues by real-time quantitative PCR

Total RNA was extracted from the chicken liver, adipose tissue, diencephalon, anterior pituitary gland, kidney, ovary, and spleen as described above. One microgram of total RNA was reverse transcribed using $d(T)_{30}$A/G/C A/G/C/T, 2U Powerscript reverse transcriptase (Clontech) in a 20 μl reaction. Chicken AdipoR1 mRNA or AdipoR2 mRNA and chicken β-actin mRNA were quantified utilizing 2 μl of the reverse transcription reaction (equivalent to 100 ng single stranded cDNA) as template in the real-time quantitative PCR. A 149-bp product for chicken AdipoR1 cDNA corresponding to nucleotides 501–649 (GenBank Accession no. DQ072275) was amplified using the following primers: forward-ccaggagaaggttgtgtttg; reverse-tgatcagcagtgcaattcct. For quantifying AdipoR2 mRNA, a 145 bp long partial AdipoR2 cDNA corresponding to nucleotides 589–733 (GenBank Accession no. DQ072276) was amplified using the following primers: forward-tcatggctcttccacacagt; reverse-aaggctgagggttgcagtag. Similarly a 123 bp product of chicken β-actin corresponding to nucleotides 1026–1148 (GenBank Accession no. L08165) was amplified using the following primer set: forward-CTGGCACCTAGCACAATGAA; reverse-CTGCTTGCTGATCCACATCT. The real time-quantitative PCR consisted of 1× Platinum SYBR® Green qPCR Super Mix-UDG (Invitrogen) and 300 nM of forward and reverse primers. The reactions were carried out in the DNA engine Opticon II (MJ Research) with the following thermocycle: 50 °C for 2 min, 95 °C for 2 min, followed by 45 cycles of 95 °C for 15 s, 55 °C for 30 s, and 72 °C for 30 s. At the end of amplification, a melting curve analysis was done by heating the PCR products from 65 to 95 °C, held for 15 s at increments of 0.2 °C and the fluorescence was detected to confirm the presence of a single amplification product. Tissue samples from each animal were run in duplicate to obtain aver-

age C_T values for AdipoR1 or AdipoR2 mRNA and β-actin mRNA. For negative controls, reverse transcription reactions using 1 μg total RNA with no reverse transcriptase (No-RT control) were used as template in place of single stranded cDNA in the real-time quantitative PCR. The log-linear threshold values (C_T) during the exponential phase of the PCR for AdipoR1 or AdipoR2 mRNA were subtracted from that of β-actin mRNA. AdipoR1 or AdipoR2 mRNA quantity was expressed as a proportion of β-actin mRNA quantity following $2^{-\Delta\Delta C_T}$ method for converting log-linear C_T values to linear term [19]. The relative amounts of AdipoR1 mRNA or AdipoR2 mRNA in various tissues were then compared.

2.5. Effect of feed deprivation on AdipoR1/AdipoR2 mRNA expression

Chickens (75-week-old leghorn Hyline W-36 strain) were either fed ad libitum or feed deprived ($n = 6$) for 48 h and killed by decapitation. Liver, adipose tissue from the abdominal fat pad, and anterior pituitary gland were collected from experimental chickens, frozen in liquid nitrogen, and stored at −80 °C until analyzed. Brains from the fed and fasted chickens were removed from the cranium, incised coronally at the level of mid-brain, frozen on powdered dry ice, and stored at −80 °C. Using a cryostat (Richard-Allen Scientific, Kalamazoo, MI), brain tissue sections (16 μm) in the coronal plane covering the entire diencephalon were made and collected in a tube. Total RNA from the diencephalon tissue sections, liver, and adipose tissue was extracted using Trizol (Invitrogen). Total RNA from the anterior pituitary gland was extracted using RNeasy kit (Qiagen). One microgram of DNAse-I treated total RNA from each tissue was reverse transcribed as described above. AdipoR1 mRNA, AdipoR2 mRNA, and β-actin mRNA quantities were determined by real time quantitative RT-PCR using SYBR® Green dye as described above. The amount of AdipoR1 mRNA or AdipoR2 mRNA was expressed as a proportion to β-actin mRNA as described above and compared between fed and fasted chickens.

2.6. Statistical analyses and softwares used

Relative AdipoR1 or AdipoR2 mRNA quantity to β-actin mRNA quantity was first converted from log-linear to linear term and then compared using a non-parametric Mann–Whitney *U*-test using SAS (SAS Institute, Cary, NC). AdipoR1 mRNA or AdipoR2 mRNA quantity in adipose tissue, liver, anterior pituitary gland, and diencephalon between fed and fasted chickens were compared using multiple Student's *t*-test. A probability level of $P < 0.05$ was considered statistically significant. For DNA sequence analysis and for PCR primer design, Vector NTI suite 9.1 (Invitrogen) was used.

3. Results

3.1. Cloning of chicken AdipoR1 and AdipoR2 cDNA

AdipoR1 and AdipoR2 cDNAs were cloned from chicken adipose tissue and the nucleotide sequences have been deposited in the GenBank (Accession nos. DQ072275, DQ072276). The open reading frame of chicken AdipoR1 and AdipoR2 cDNAs consisted of 1128 and 1161 bp, respectively. The chicken AdipoR1 cDNA and AdipoR2 cDNA

Table 1
Comparison of chicken AdipoRl and AdipoR2 with mammalian AdipoRl and AdipoR2

Species	GenBank Accession no.	Length (bp)	Nucleotide % identity	Amino acid % identity
(a) AdipoR1				
Human	NM_015999	1128	80	91
Mouse	NM_028320	1128	82	91
Rat	NM_207587	1128	82	91
Pig	NM_001007193	1128	83	91
(b) AdipoR2				
Human	NM_024551	1161	76	82
Mouse	NM_197985	1161	76	82
Pig	AY606803	1161	78	82

sequences were only 68% homologous to each other, a common property with mammalian AdipoR1 and AdipoR2 cDNA sequences [12]. However, the chicken AdipoR1 cDNA was found to be 80–83% homologous to human, mouse, rat, or pig AdipoR1 cDNA, while the deduced protein sequence was 91% similar to mammalian AdipoR1 (Table 1). Similarly, the chicken AdipoR2 cDNA was 76–78% homologous to human, mouse, or pig AdipoR2 cDNA, while the deduced protein sequence was 82% similar to mammalian AdipoR2 (Table 1). A hydrophobicity plot analysis [20] of the deduced amino acid sequences of the chicken AdipoR1 and AdipoR2 cDNAs revealed a series of seven highly hydrophobic regions as found in mammalian AdipoR1 and AdipoR2 [12] that represent transmembrane regions of the receptor.

3.2. *Genomic locus of AdipoR1 or AdipoR2*

A BLAST search using the first draft of the chicken genome project (http://www.ensembl.org/Gallus_gallus/blastview/ [21]) was done to identify the genomic location of AdipoR1 and AdipoR2 cDNA cloned in the present study. The coding nucleotide sequence of AdipoR1 was located on chromosome 32 (nt 20578–25859), while that of AdipoR2 was located on chromosome 1 (nt 57603978–57624369).

3.3. *RT-PCR*

Partial cDNAs corresponding to nucleotides 389–738 (GenBank Accession no. DQ072275) for AdipoR1 mRNA or nucleotides 430–774 (GenBank Accession no. DQ072276) for AdipoR2 mRNA were amplified from the single stranded cDNA reverse transcribed from total RNA extracted from chicken adipose tissue, liver, diencephalon, skeletal muscle, kidney, spleen, ovary, anterior pituitary gland, and blood (Fig. 1). The nucleotide sequence of the AdipoR1 or AdipoR2 RT-PCR products was identical to the region of chicken AdipoR1 or AdipoR2 cDNA from where they were amplified. In addition, a BLAST search of the chicken AdipoR1/AdipoR2 RT-PCR products revealed significant homology only to the respective mammalian sequences and showed no homology to any other chicken genes. Reverse transcription reactions consisting of total RNA from each

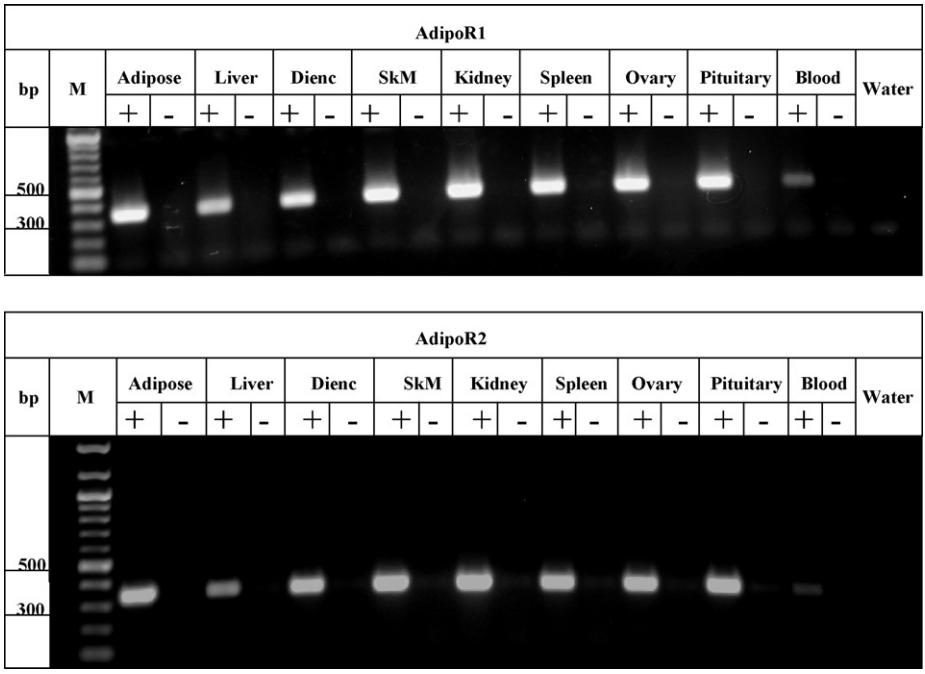

Fig. 1. RT-PCR analysis of AdipoR1 or AdipoR2 gene expression in various tissues in the chicken ($n = 4$). Total RNA extracted from each tissue was DNAse-I digested and reverse transcribed. Approximately 100 ng of cDNA (+) was used as template to amplify a 350 bp (AdipoR1) or a 345 bp (AdipoR2) product. Contamination controls consisted of RNA from each tissue without reverse transcriptase (−) or substitution of water for the template. M: Molecular weight standard; bp: base pair; Dienc: diencephalon; SkM: skeletal muscle.

tissue but without reverse transcriptase did not yield any product, suggesting that genomic DNA did not contribute for amplification of the AdipoR1 or AdipoR2 PCR product (Fig. 1). Presence of AdipoR1 and AdipoR2 mRNA transcripts in the blood may have contributed to the AdipoR1 and AdipoR2 gene expression in other tissues studied as blood contamination in the tissues cannot be avoided. However, we detected robust expression of the AdipoR1 and AdipoR2 genes in a chicken liver hepatoma cell line that represents a pure population of hepatocytes devoid of blood and connective tissue cells, indicating that hepatocytes do express the AdipoR1 and AdipoR2 genes (Ramachandran and Vasilaltos-Younken, unpublished observation). Similarly, a recent study utilizing in situ hybridization histochemistry confirmed the presence of AdipoR1 and AdipoR2 mRNA transcripts in several tissues in the mouse embryo [22].

3.4. Relative quantity of AdipoR1 and AdipoR2 mRNA in various tissues

Skeletal muscle, adipose tissue, and diencephalon was found to contain the highest quantities of AdipoR1 mRNA followed by kidney, ovary, liver, anterior pituitary, and spleen (Fig. 2A). Adipose tissue had the highest quantity of AdipoR2 mRNA followed by skeletal muscle, liver, ovary, diencephalon, anterior pituitary, kidney, and spleen (Fig. 2B). Melting

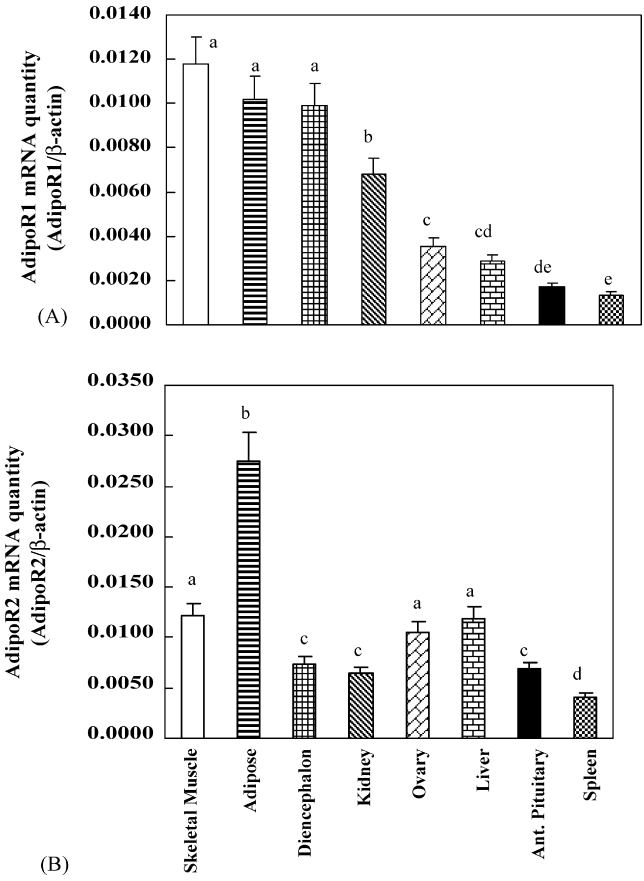

Fig. 2. AdipoR1 (A) or AdipoR2 (B) mRNA quantity relative to β-actin mRNA quantity in various tissues in the chicken. Total RNA from each tissue was DNAse-I treated and reverse transcribed. Approximately 100 ng of cDNA was used in real-time quantitative PCR using SYBR® green as the dye to quantitate AdipoR1 mRNA, AdipoR2 mRNA, or β-actin mRNA in separate reactions. Each reaction was run in duplicate and threshold (C_T) values for AdipoR1 mRNA or AdipoR2 mRNA were subtracted from that of β-actin mRNA and converted from log-linear to linear term. The data represents mean values from four chickens for each tissue. Data with *different letters* above each bar within each chart represents significant difference at $P < 0.05$.

curve analyses showed the presence of a single PCR product for AdipoR1, AdipoR2, or β-actin, confirming the specificity of the reaction (data not shown).

3.5. Effect of feed deprivation on AdipoR1 mRNA or AdipoR2 mRNA quantity

Feed deprivation for 48 h resulted in a significant decrease ($P < 0.05$) in AdipoR1 mRNA quantity in the anterior pituitary gland but not in adipose tissue, diencephalon, and liver (Fig. 3A). Similarly, AdipoR2 mRNA content was significantly increased ($P < 0.05$) in adipose tissue due to a 48 h feed deprivation, while its quantity remained unchanged in liver, diencephalon, and anterior pituitary gland (Fig. 3B). Melting curve analyses showed

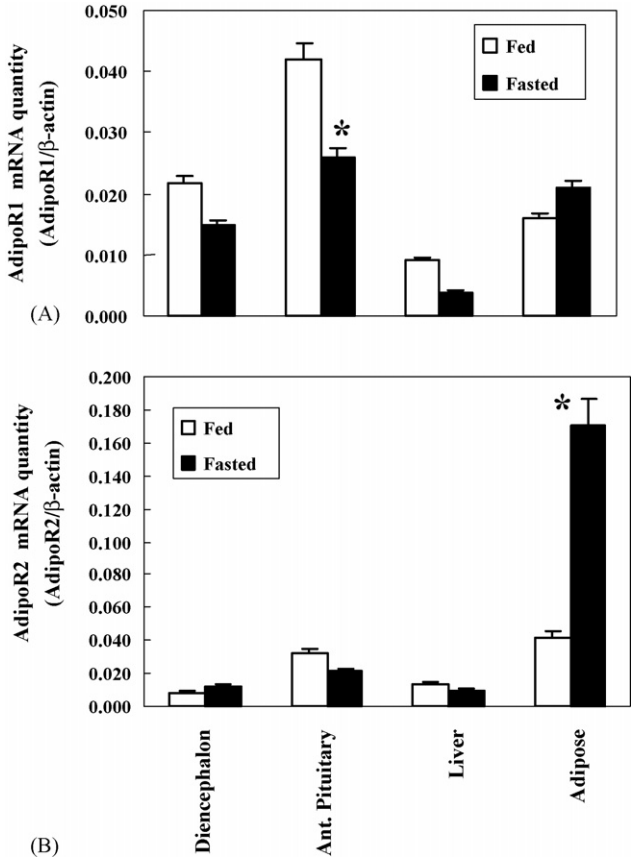

Fig. 3. Effect of feed deprivation on AdipoR1 mRNA (A) or AdipoR2 mRNA (B) quantity in the chicken diencephalon, anterior pituitary gland, liver and adipose tissue. Chickens ($n = 6$) were fed ad libitum or fasted for 48 h and killed by decapitation. Total RNA was extracted from tissues and DNAse-I treated. Following reverse transcription, approximately 100 ng of cDNA was used in real-time quantitative PCR using SYBR® green as the dye to quantitate AdipoR1 mRNA, AdipoR2 or β-actin mRNA in separate reactions from fed and fasted chickens. Each reaction was run in duplicate and the threshold (C_T) values for AdipoR1 mRNA were subtracted from that of β-actin mRNA, averaged and converted from log-linear to linear term. An asterisk above the bar represents significant difference at $P < 0.05$.

the presence of a single PCR product for AdipoR1, AdipoR2, or β-actin product confirming the specificity of the reaction (data not shown).

4. Discussion

This is the first report on cloning chicken AdipoR1 and AdipoR2 cDNA sequences. The cDNAs encoding chicken AdipoR1 and AdipoR2 shared high degree of homology with the respective mammalian homologues suggesting that both genes are evolutionarily conserved and may have important biological functions. In mammalian species, AdipoR1

was reported to have greater affinity for the globular domain of adiponectin while AdipoR2 has preferential binding to full length adiponectin comprising of collagenous and globular domains. The predicted sequence of chicken adiponectin contains putative globular and collagenous domains [16] as found in mammalian species. However, relative affinities of the chicken AdipoR1 and AdipoR2 to adiponectin fractions remain to be determined. Nevertheless, the presence of two receptors for adiponectin in the chicken as found in mammalian species may indicate similarity in biological functions. Furthermore, hydrophobicity analyses of the deduced amino acid sequence of the chicken AdipoR1 and AdipoR2 revealed seven hydrophobic regions representing seven transmembrane domains. Again, this pattern is similar to mammalian AdipoR1 and AdipoR2 that have also been predicted to contain seven transmembrane domains [12].

We found that the AdipoR1 and AdipoR2 genes are expressed in multiple tissues in the chicken wherein both receptors are likely to mediate the physiological effects of adiponectin. In addition, chicken AdipoR1 and/or AdipoR2 may also regulate de novo expression of the adiponectin gene in these tissues [16] in an autocrine and/or paracrine regulatory pathway. We determined the relative expression of AdipoR1 and AdipoR2 mRNA in various tissues using real time quantitative PCR and found that skeletal muscle, adipose tissue and diencephalon were the principal organs where AdipoR1 gene was maximally expressed, while AdipoR2 mRNA expression was the highest in adipose tissue. In contrast to our findings in the chicken, mouse AdipoR1 and AdipoR2 mRNAs have been found to be maximally expressed in the skeletal muscle and liver, respectively [12]. This may represent species variation in the gene expression of AdipoR1 and AdipoR2.

This is the first report describing robust expression of AdipoR1 mRNA in the brain of any species. In the past, low levels of AdipoR1 and AdipoR2 mRNA have been detected in the mouse brain [12]. AdipoR1 expression in the diencephalon may suggest its involvement in adiponectin signaling possibly in the hypothalamus. In this regard, intracerebroventricular administration of adiponectin has been found to affect energy metabolism and body weight in mice [23].

The quantities of Adipo2 mRNA in other tissues were highest in liver, skeletal muscle, and ovary followed by diencephalon, anterior pituitary gland, kidney, and spleen. The liver is the primary site of lipid biosynthesis [24] in avian species unlike rodents where fat synthesis occurs in white adipose tissue. AdipoR2 mRNA expressed in the chicken liver may possibly be involved in lipid metabolism, glucose utilization, and gluconeogenesis. Adiponectin receptors (both AdipoR1 and AdipoR2) expressed in skeletal muscle are possibly involved in glucose utilization as in mammals and also in thermogenesis as chicken skeletal muscle fast twitch fibers convert to slow twitch fibers for heat production when exposed to cold [25]. It is tempting to speculate that both AdipoR1 and AdipoR2 mRNA expressed in the ovary are possibly involved in steroid synthesis as it was recently shown that AMPK, a molecule that is activated by adiponectin, is involved in ovarian progesterone synthesis [26].

This is the first report on documenting AdipoR1 and AdipoR2 gene expression in the anterior pituitary gland in any species. AdipoR1 and AdipoR2 found in the chicken pituitary gland may possibly regulate pituitary hormone secretion. In this regard, prolactin and growth hormone have been found to affect AdipoR1 and AdipoR2 gene expression in adipose tissue [27], and plasma growth hormone concentrations are inversely related to adiponectin concentrations in mice [28] and in humans [29].

We earlier reported that fasting in chickens significantly decreased the adiponectin gene expression in adipose tissue, liver, anterior pituitary gland but not in diencephalon [16]. Feed deprivation is likely to create a negative energy balance that may have altered expression of the AdipoR1 or AdipoR2 genes in these organs involved in metabolism or neuroendocrine system. In the present study, we found that a 48 h feed deprivation significantly increased AdipoR2 mRNA quantity in the adipose tissue and decreased AdipoR1 mRNA quantity in the anterior pituitary gland with no change in liver and diencephalon AdipoR1 or AdipoR2 mRNA quantity. A significant change in AdipoR1 or AdipoR2 mRNA quantity and a concomitant decrease in adiponectin mRNA quantity in response to fasting suggest that adiponectin may possibly regulate its own secretion from adipose tissue and/or from pituitary gland in an autocrine and/or paracrine regulatory pathway. In this regard, food deprivation in mice leads to increased AdipoR1 and AdipoR2 mRNA expression in liver and skeletal muscles and refeeding restored the AdipoR1 and AdipoR2 gene expression to a level equal to the original fed state [30]. AdipoR1 mRNA concentration in adipose tissue was not affected by short term feed restriction in pigs while that of AdipoR2 was significantly increased [31] similar to our findings in the chicken. Expression of both AdipoR1 and AdipoR2 genes in mice appears to be inversely regulated by insulin in physiological and pathophysiological states such as fasting in mice [30]. A significant increase in AdipoR2 gene expression in adipose tissue due to fasting in the chicken may possibly be due to decreased plasma insulin levels that normally occur during fasting in the chicken [32]. In contrast, a decrease in AdipoR1 gene expression in the pituitary gland may suggest that its gene expression is apparently not under the control of insulin. This conclusion is supported by a report that insulin did not directly modify AdipoR1 mRNA expression in human skeletal muscle cells cultured in vitro [33]. Although expression of the AdipoR1 or AdipoR2 genes fluctuates in response to feeding status, it remains to be determined whether such changes are reflected in AdipoR1 or AdipoR2 protein expression.

In summary, we have cloned the chicken AdipoR1 and AdipoR2 cDNA and found that both genes are ubiquitously expressed in multiple tissues of the chicken. In addition, we found that both AdipoR1 and AdipoR2 gene expression in the chicken anterior pituitary gland and adipose tissue are significantly altered by feed deprivation. Further studies are required to characterize the functional role of AdipoR1 and AdipoR2 in metabolism and energy homeostasis in the chicken.

Acknowledgement

This project was supported in part by National Research Initiative Competitive Grant no. 2004-35203-14755 from the USDA Cooperative State Research, Education, and Extension Service to RR.

References

[1] Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem 1995;270:26746–9.

[2] Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). Biochem Biophys Res Commun 1996;221:286–9.
[3] Hu E, Liang P, Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem 1996;271:10697–703.
[4] Nakano Y, Tobe T, Choi-Miura NH, Mazda T, Tomita M. Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma. J Biochem (Tokyo) 1996;120:803–12.
[5] Pajvani UB, Du X, Combs TP, Berg AH, Rajala MW, Schulthess T, et al. Structure–function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications fpr metabolic regulation and bioactivity. J Biol Chem 2003;278:9073–85.
[6] Tsao TS, Lodish HF, Fruebis J. ACRP30, a new hormone controlling fat and glucose metabolism. Eur J Pharmacol 2002;440:213–21.
[7] Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, et al. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Sci USA 2001;98:2005–10.
[8] Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002;8:1288–95.
[9] Winder WW, Hardie DG. AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. Am J Physiol 1999;277:E1–10.
[10] Tomas E, Tsao TS, Saha AK, Murrey HE, Zhang Cc C, Itani SI, et al. Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. Proc Natl Acad Sci USA 2002;99:16309–13.
[11] Ruderman NB, Saha AK, Vavvas D, Heydrick SJ, Kurowski TG. Lipid abnormalities in muscle of insulin-resistant rodents. The malonyl CoA hypothesis. Ann N Y Acad Sci 1997;827:221–30.
[12] Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 2003;423:762–9.
[13] Chinetti G, Zawadski C, Fruchart JC, Staels B. Expression of adiponectin receptors in human macrophages and regulation by agonists of the nuclear receptors PPARalpha, PPARgamma, and LXR. Biochem Biophys Res Commun 2004;314:151–8.
[14] Berner HS, Lyngstadaas SP, Spahr A, Monjo M, Thommesen L, Drevon CA, et al. Adiponectin and its receptors are expressed in bone-forming cells. Bone 2004;35:842–9.
[15] Kharroubi I, Rasschaert J, Eizirik DL, Cnop M. Expression of adiponectin receptors in pancreatic beta cells. Biochem Biophys Res Commun 2003;312:1118–22.
[16] Maddineni S, Metzger S, Ocon O, Hendricks 3rd G, Ramachandran R. Adiponectin gene is expressed in multiple tissues in the chicken: food deprivation influences adiponectin messenger ribonucleic acid expression. Endocrinology 2005;146:4250–6.
[17] Koike TI, Nalbandov AV, Dimick MK, Matsumura Y, Lepkovsky S. Action of insulin upon blood glucose levels of fasted hypophysectomized. Depancreatized and normal chickens. Endocrinology 1964;74: 944–8.
[18] Herzberg GR, Rogerson M. Tissue distribution of lipogenesis in vivo in the common murre (*Uria aalge*) and the domestic chicken (*Gallus domesticus*). Comp Biochem Physiol B 1990;96:767–9.
[19] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001;25:402–8.
[20] Kyte J, Doolittle RF. A simple method for displaying the hydropathic character of a protein. J Mol Biol 1982;157:105–32.
[21] Wallis JW, Aerts J, Groenen MA, Crooijmans RP, Layman D, Graves TA, et al. A physical map of the chicken genome. Nature 2004;432:761–4.
[22] Zhou Y, Sun X, Jin L, Stringfield T, Lin L, Chen Y. Expression profiles of adiponectin receptors in mouse embryos. Gene Expr Patterns 2005;5:711–5.
[23] Qi Y, Takahashi N, Hileman SM, Patel HR, Berg AH, Pajvani UB, et al. Adiponectin acts in the brain to decrease body weight. Nat Med 2004;10:524–9.
[24] Leveille GA, Romsos DR, Yeh Y, O'Hea EK. Lipid biosynthesis in the chick. A consideration of site of synthesis, influence of diet and possible regulatory mechanisms. Poult Sci 1975;54:1075–93.

[25] Hirabayashi M, Ijiri D, Kamei Y, Tajima A, Kanai Y. Transformation of skeletal muscle from fast- to slow-twitch during acquisition of cold tolerance in the chick. Endocrinology 2005;146:399–405.
[26] Tosca L, Froment P, Solnais P, Ferre P, Foufelle F, Dupont J. Adenosine 5′-monophosphate-activated protein kinase regulates progesterone secretion in rat granulosa cells. Endocrinology 2005;146:4500–13.
[27] Nilsson L, Binart N, Bohlooly YM, Bramnert M, Egecioglu E, Kindblom J, et al. Prolactin and growth hormone regulate adiponectin secretion and receptor expression in adipose tissue. Biochem Biophys Res Commun 2005;331:1120–6.
[28] Berryman DE, List EO, Coschigano KT, Behar K, Kim JK, Kopchick JJ. Comparing adiposity profiles in three mouse models with altered GH signaling. Growth Horm IGF Res 2004;14:309–18.
[29] Lam KS, Xu A, Tan KC, Wong LC, Tiu SC, Tam S. Serum adiponectin is reduced in acromegaly and normalized after correction of growth hormone excess. J Clin Endocrinol Metab 2004;89:5448–53.
[30] Tsuchida A, Yamauchi T, Ito Y, Hada Y, Maki T, Takekawa S, et al. Insulin/Foxo1 pathway regulates expression levels of adiponectin receptors and adiponectin sensitivity. J Biol Chem 2004;279:30817–22.
[31] Ding ST, Liu BH, Ko YH. Cloning and expression of porcine adiponectin and adiponectin receptor 1 and 2 genes in pigs. J Anim Sci 2004;82:3162–74.
[32] Krestel-Rickert DH, Baile CA, Buonomo FC. Changes in insulin, glucose and GH concentrations in fed chickens. Physiol Behav 1986;37:361–3.
[33] Staiger H, Kaltenbach S, Staiger K, Stefan N, Fritsche A, Guirguis A, et al. Expression of adiponectin receptor mRNA in human skeletal muscle cells is related to in vivo parameters of glucose and lipid metabolism. Diabetes 2004;53:2195–201.