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Expression profiles of three isoforms of inositol 1,4,5-trisphosphate receptor in brown adipose tissue of the rat

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Abstract

The thermogenic function of brown adipose tissue (BAT) is known to be mainly regulated by a signal transduction cascade *via* β-adrenoceptor. However, recent studies indicated that the α-adrenoceptor and its downstream signal transduction cascade, causing elevation of the cellular Ca^{2+} level, are also important for the regulation of this function of BAT. In the present study, expression profiles of 3 isoforms of the inositol 1,4,5-trisphosphate (IP_3) receptor, known as one of the major components of the machinery regulating the intracellular Ca^{2+} concentration in the BAT of rats, were investigated by Northern analysis. Of these three IP_3 receptor isoforms, the type 2 one was found to be the most abundant of the three in BAT. Furthermore, when rats were exposed to the cold, under which condition the thermogenic function of BAT is known to be stimulated, the expression levels of types 1 and 2 isoforms of IP_3 receptor were remarkably elevated. The results of Western analysis supported the predominant expression of the type 2 isoform in BAT. However, different from the results of Northern analysis, the expression levels of types 1 and 2 isoforms of IP_3 receptor protein in BAT were not influenced by exposure of the animals to the cold.

Keywords: Brown adipose tissue; Energy expenditure; IP_3 receptor; Northern analysis; Western analysis

1. Introduction

The energy expenditure function of BAT is known to be attributable to the action of the type 1 uncoupling protein specifically expressed in the mitochondria of this tissue. This protein dissipates the electrochemical potential difference of H^+ ($\Delta\mu H^+$) across the inner mitochondrial membrane, and thus stimulates oxidation of respiratory substrates without the accompanied formation of ATP. As a result, the expenditure of excess energy and effective thermogenesis can be achieved (for reviews, see [1,2]).

This energy expenditure function of BAT is known to be elevated under certain conditions such as a cold environment. Signals causing elevation of the thermogenic activity of the BAT have been well shown to be mainly transduced by NE as a neurotransmitter. However, at least two α-adrenoceptors (α-ARs) and three β-ARs are known to be responsible for the signal of NE; and these ARs are widely expressed throughout the tissues. Of these, the type 3 β-adrenoceptor (β_3-AR) is specifically expressed in adipose tissues [3,4], and believed to be responsible for the transduction of the stimulatory signal of NE in BAT. The binding of NE to β_3-AR causes elevation of the cellular cyclic AMP level and subsequent stimulation of protein kinase A. As a result, the transcription of genes responsible for the thermogenic activity of the BAT is accelerated.

In addition to the cascade responsible for transduction of the NE signal *via* β_3-AR, the importance of the elevation of the Ca^{2+} concentration in brown adipocytes for the elevation of thermogenic activity of the BAT, possibly resulting from the transduction of the NE signal *via* the type 1

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Abbreviations: AR, adrenoceptor; BAT, brown adipose tissue; IP_3, inositol 1,4,5-trisphosphate; NE, norepinephrine.

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α-adrenoceptor (α_1-AR), was also reported [5–9]. Upon the binding of NE to α_1-AR, receptor associated G-protein activates phospholipase C. This activated phospholipase C stimulates the hydrolysis of phosphatidylinositol bisphosphate and forms diacylglycerol and IP_3. The latter binds to the IP_3 receptor expressed on the surface of the endoplasmic reticulum and causes elevation of the intracellular Ca^{2+} concentration. However, the physiological relevance of the latter cascade to the thermogenic activity of the BAT is still uncertain.

Three isoforms of the IP_3 receptor are expressed in mammals. Their structural properties and expression profiles in various tissues are well established (for reviews, see [10,11]); however, their expression profiles in BAT had not yet been examined. As described above, the possible involvement of α_1-AR and subsequent signal transduction cascade in the regulation of the thermogenic activity of BAT were reported; and, therefore, it is very interesting and of importance to examine the expression profiles of IP_3 receptors in BAT. In this study, we investigated the expression profiles of the three isoforms of IP_3 receptor in BAT of the rat.

2. Materials and methods

2.1. Preparation of probes of the three IP_3 receptor isoforms

cDNA fragments corresponding to the 3′ regions of messages of three IP_3 receptor isoforms of rats were prepared by RT-PCR with the following amplimers: MB238 (5′-atggtgacttgtcactcctg) and MB239 (5′-gacttgcttcagaactctgc) for type 1, MB240 (5′-taaactctcagccaccaagg) and MB241 (5′-tgtgatgcagaatggagcac) for type 2, and MB242 (5′-ggagagggacaagtttgaca) and MB243 (5′-agagaagccactgtcacaca) for type 3 isoform. Amplified regions were nucleotides 8772–9802 [12], 9579–10650 [13], and 7759–8721 [14] for the respective types 1, 2, and 3 isoforms. First-strand cDNA prepared from message expressed in rat brain was used as a template for amplification by PCR.

2.2. Northern blotting

Total RNA samples were obtained from various tissues of normal male Wistar rats (4 weeks old, kept at 23°). For preparation of RNA samples from the BAT of cold-exposed rats, prior to extraction of the BAT, the animals were kept at 4° for 48 hr. To eliminate the probable differences between individual animals, tissues of four rats were pooled and used for isolation of RNA samples or for the preparation of the microsomal fraction (see below). Poly(A)$^+$ RNA was purified from total RNA by using Oligotex dT Super. Samples of 1.0 μg of poly(A)$^+$ RNAs were subjected to denatured agarose gel electrophoresis and transferred onto nitrocellulose membranes. Hybridizations were carried out according to the standard protocol. To ensure that equal amounts of RNA samples had been loaded, we also measured the hybridization signal of β-actin.

2.3. Preparation of microsomal fractions of various tissues and Western blotting

For analysis of the expression profiles of types 1 and 2 isoforms of IP_3 receptor protein, microsomal fractions were prepared from brain, liver, heart, and BAT of 4-week-old normal rats and from BAT of cold-exposed rats of the same age, according to the procedure reported previously [15]. Microsomal proteins (20 μg) were subjected to SDS-PAGE, and then transferred to a nitrocellulose membrane. Proteins corresponding to types 1 and 2 isoforms of the IP_3 receptor were detected by using their specific antibodies, obtained from Alexis Biochemicals, and Chemicon International, respectively.

3. Results and discussion

Three isoforms of the IP_3 receptor, type 1 (IP_3R1), type 2 (IP_3R2), and type 3 (IP_3R3), were identified earlier; and their tissue distributions were well investigated (for reviews, see [10,11]). Namely, IP_3R1 is highly expressed in the central nervous system, particularly in the cerebellum; IP_3R2 is expressed in many tissues, but significantly in the spinal cord and glial cells; and IP_3R3 is found in the kidney, brain, gastrointestinal tract, and pancreatic islets. However, no data were available in the literature for the expression profiles of IP_3 receptor isoforms in BAT. Thus, we compared the expression levels of the three IP_3 receptor isoforms between BAT and other tissues. Since these isoforms show relatively high structure similarity of 73–77% [10], to avoid cross hybridization of probes, we prepared cDNA fragments corresponding to the 3′ noncoding regions of each message (for details, see Section 2) and used them as specific probes.

As shown in Fig. 1, IP_3R1 was most significantly expressed in the brain. A moderate signal of this isoform was also observed in the kidney and heart, but the expression level of IP_3R1 in the liver, skeletal muscle, and BAT was very low. On the contrary, remarkable signals of IP_3R2 were observed in all RNA samples except for that sample from skeletal muscle. It is noteworthy that the hybridization signal of IP_3R2 was the most intense in BAT. Although the specific radioactivities of the probes for the three IP_3 receptors were not markedly different, signals corresponding to the type 3 isoform (IP_3R3) were very weak throughout all of the tissues analyzed, indicating a much lower expression level of this isoform in mammalian tissues. However, a faint signal of this isoform was observed in the kidney, heart, and BAT. To compare the expression levels of two (or more) distinct messages, in an exact sense, one should compare

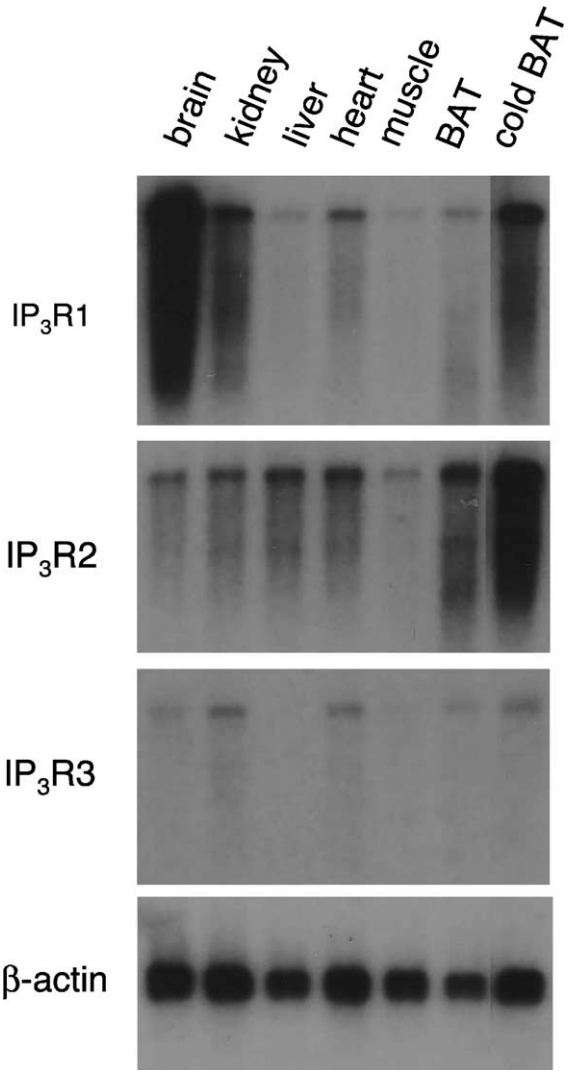

Fig. 1. Comparison of steady-state transcript levels of three IP_3 receptor isoforms between BAT and other tissues. IP_3R1, IP_3R2, and IP_3R3 represent the respective types 1, 2, and 3 isoforms of the IP_3 receptor. Transcript levels of these three IP_3 receptor isoforms in BAT were compared with those in five typical tissues. Furthermore, those in BAT of cold-exposed rat were also compared. Signal intensities of β-actin in these RNA samples were also measured as a control for equal loading. Results typical of more than three independent runs are shown.

signal intensities of the hybridization bands under normalized conditions using known amounts of synthesized mRNA [16], since specific radioactivities of probes and their affinities against their corresponding messages might be different. However, we roughly concluded that the IP_3R2 (type 2 isoform) is the major isoform of IP_3 receptor expressed in BAT, since the expression levels of the other two isoforms in BAT were almost negligible.

As stated above, the energy expenditure function of BAT is remarkably elevated when animals are exposed to cold. Thus, transcript levels of these IP_3 receptor isoforms in BAT isolated from cold-exposed rats were also examined. As a result, expression levels of both of IP_3R1 and IP_3R2 in BAT were found to be remarkably elevated when rats were exposed to the cold. It should be noted that the transcript level of IP_3R1 in the BAT of cold-exposed rats was still lower than that in the brain of control rats but that the level of IP_3R2 mRNA in the BAT of the cold-exposed animals was quite increased over that of the control BAT. On the contrary, the intensity of the faint signal corresponding to IP_3R3 in BAT was not remarkably different between rats kept at 23° and those exposed to the cold.

The IP_3 receptor is known to function in its tetrameric form in membrane systems such as endoplasmic reticulum, and was reported to exist as a heterotetramer of distinct isoforms [17]. Studies on the question as to how the functional properties of various heterotetramers of the IP_3 receptor differ from each other have already started to appear [18]; however, the physiological meanings of the heterogeneous status of IP_3 receptor are not yet fully established. Thus, to understand the roles of IP_3 receptor isoforms in BAT, analysis of expression levels of these proteins is very important. Thus, we next examined the expression profiles of IP_3R1 and IP_3R2 proteins in various tissues. As a result, as shown in Fig. 2, IP_3R1 was significantly expressed in the brain. Although the expression of this isoform in the liver and heart was almost negligible, a definite signal of this isoform was also observed in BAT. On the contrary, IP_3R2 was most significantly expressed in BAT. Weaker signals were also observed in brain and liver, but the signal of this isoform in the heart was almost negligible. Thus, the predominant expression of IP_3R2 in BAT was also confirmed at the protein level. However, different from the results of Northern analysis, expression levels of IP_3R1 and IP_3R2 proteins in BAT were not influenced by cold exposure.

As stated above, recent studies indicated the existence of an additional cascade stemming from α_1-AR and leading to an elevated cellular Ca^{2+} concentration, and this cascade was also considered to be important for the regulation of the thermogenic function of BAT [5–9]. The involvement of the IP_3R in the signal transduction in the downstream of

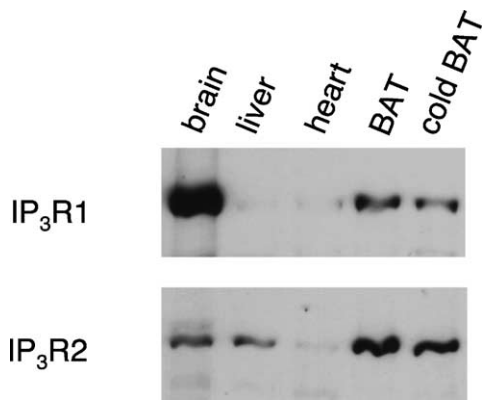

Fig. 2. Western analysis of types 1 and 2 IP_3 receptors expressed in various rat tissues. Microsomal proteins (20 μg) prepared from brain, liver, heart, and BAT of normal rats, and from the BAT of cold-exposed rats were subjected to SDS-PAGE and transferred to a nitrocellulose membrane. Proteins of types 1 and 2 IP_3 receptors were detected with their specific antibodies. Results typical of more than three independent runs are shown.

α-AR has been well established. However, until recently, there was no description regarding the roles of IP_3R in the thermogenic function of BAT. Thus, to understand how the energy-dissipating function of BAT is regulated, studies on the expression profiles of IP_3R in BAT assume much importance.

In the present study, we evaluated expression levels of the three IP_3R isoforms in BAT by both Northern and Western analyses. As a result, the type 2 isoform (IP_3R2) was concluded to be the major isoform of the IP_3 receptor expressed in BAT. Although the results of Northern analysis showed significant elevation of the message levels of IP_3R1 and IP_3R2 in the BAT of animals exposed to the cold, the Western analysis showed that expression levels of these proteins in BAT were insensitive to cold-exposure. A simple interpretation of this discrepancy is as follows: at least under the experimental conditions used, changes in the expression levels of IP_3R1 and IP_3R2 proteins in the BAT of cold-exposed rats were not physiologically required; however, elements responsive to stimulation by cold-exposure may be present in the regulatory regions of the genes encoding these IP_3 receptors. To confirm the validity of this interpretation, and to understand in greater detail how the energy metabolism in BAT is regulated, we are now conducting further studies.

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