**Effects of β₂-agonists and exercise on β₂-adrenergic receptor signaling in skeletal muscles**

Shogo Sato¹,²*, Ken Shirato¹, Takako Kizaki³, Hideki Ohno³, Kaoru Tachiyashiki⁴ and Kazuhiko Imaizumi¹,⁵**

¹Laboratory of Physiological Sciences, Faculty of Human Sciences, Waseda University, 2-579-15 Mikajima, Tokorozawa, Saitama 359-1192, Japan
²Japan Society for the Promotion of Science, 8 Ichiban-cho, Chiyoda-ku, Tokyo 102-8472, Japan
³Department of Molecular Predictive Medicine and Sport Science, Kyorin University, School of Medicine, 6-20-2 Shinkawa, Mitaka, Tokyo 181-8611, Japan
⁴Department of Natural and Living Sciences, Graduate School of Education, Joetsu University of Education, 1 Yamayashiki, Joetsu, Niigata 943-8512, Japan
⁵Global COE Doctoral Program, Graduate School of Sport Sciences, Waseda University, 2-579-15 Mikajima, Tokorozawa, Saitama 359-1192, Japan

Received: February 23, 2012 / Accepted: February 27, 2012

**Abstract** In this review, we discuss the anabolic and metabolic responses of skeletal muscles to β₂-agonists and exercise. β₂-agonists increase muscle mass, particularly in fast-twitch muscles. Exercise positively regulates glucose homeostasis, mitochondrial biogenesis, and metabolic enzyme levels in skeletal muscles; whereas treatment with β₂-agonists attenuates these beneficial effects. This review also describes the role of β₂-adrenergic receptor (β₂-AR) signaling molecules, such as cyclic adenosine monophosphate response element-binding protein, mitogen-activated protein kinase, and Akt/protein kinase B, in the response of skeletal muscles to β₂-agonist treatment and exercise. For example, β₂-agonists and exercise increase the phosphorylation of p38 mitogen-activated protein kinase in slow-twitch muscles. Our interpretation of these findings is that β₂-adrenergic receptor signaling plays a functional role in the anabolic and metabolic responses of skeletal muscles to β₂-agonists and exercise.

**Keywords**: Sports doping, β₂-agonist clenbuterol, Exercise, Skeletal muscle, β₂-adrenergic receptor signaling

**Introduction**

Skeletal muscles can adapt to a range of different physiological challenges. For example, resistance training increases muscle mass, fiber hypertrophy, and strength⁶. In contrast, endurance training results in increased mitochondrial density, capillary density, changes in key metabolic enzyme levels, and increased maximal oxygen uptake²³. These adaptations of skeletal muscles to exercise are mediated, at least in part, by β₂-adrenergic receptors (β₂-ARs)⁸. The β₂-AR, a member of the guanine nucleotide-binding G protein-coupled receptor (GPCR) family, has 7 transmembrane α helices forming 3 extracellular loops, including an NH₂ terminus and 3 intracellular loops that include a COOH terminus⁹. Skeletal muscles contain a significant proportion of β-ARs, of which the β₂ subtype is the most abundant, while approximately 7-10% of β-ARs are of the β₁ subtype⁶. Furthermore, β₂-AR is more expressed in slow-twitch fibers than in fast-twitch fibers⁷ and is often a pharmacological target for performance-enhancing drugs in sports.

In modern sports, many types of doping drugs have been used by athletes to improve athletic performance despite many negative reactions and side effects⁸⁹. β₂-agonists, such as clenbuterol, salbutamol, and fenoterol (Fig. 1), increase muscle mass and power⁸⁹. According to the recent reports by the World Anti-Doping Agency, the β₂-agonist clenbuterol was the seventh most commonly used anabolic agent in 2009 (67 cases; 2.0% of all anabolic agents used).

The role of β₂-AR signaling in the regulation of protein metabolism in skeletal muscles remains to be elucidated. Understanding the mechanism of adaptation of skeletal muscles to β₂-AR activation could lead to the eradication of its use in sports doping. Thus, in this review, we dis-
Effects of endurance training, such as contractile activity

formation of slow-twitch muscles to fast-twitch muscles

hypertrophic action, clenbuterol also induces the trans-

Numerous studies have shown that β-agonists mediate the fiber type shift in skeletal muscles. However, the mechanisms by which β-agonists exert fiber type-dependent effects on muscle hypertrophy remain unclear.

In general, physical activity elicits physiological responses in skeletal muscles and in turn results in numerous health benefits. In the acute state, exercise positively regulates glucose homeostasis by enhancing glucose transport and insulin action in skeletal muscles. Chronic physical activity increases glucose transporter 4 protein levels and mitochondrial enzyme expression, and modifies the fiber type in skeletal muscles. However, supplementing strength training with clenbuterol treatment has no synergistic benefit on the development of muscle mass or metabolic enzyme levels.

In addition to strength training, β-agonists also offset the beneficial effects of endurance training, such as contractile activity;

Effects of β-agonists and exercise on the Ga-adenyl cyclase (AC)-cyclic adenosine monophosphate (cAMP) pathway in skeletal muscles

It is known that β-AR can couple to the Ga and Go signaling pathways (Fig. 2). Ga-AC-cAMP pathway activation stimulates phosphorylation of the C subunit of protein kinase A (PKA), as illustrated in Fig. 2. Phosphorylated PKA regulates the activity of several proteins, including CRE response element (CRE)-binding protein (CREB), suggesting that the response of skeletal muscles to β-agonists and exercise is not primarily dependent upon the activation of CREB. From these findings, it may be concluded that the Ga-AC-cAMP pathway is not activated by β-agonists or exercise-induced β-AR stimulation.

Effects of β-agonists and exercise on mitogen-activated protein kinase (MAPK) pathway in skeletal muscles

In addition to its well-documented inhibition of AC activity, β-AR coupling to Go appears to activate Ga-independent pathways (Fig. 2). In one such pathway, the receptor-coupled Ga, dissociates from the heterodimeric Gβγ complex, and free Gβγ subunits then activate the MAPK signaling pathway. The MAPK family, including the extracellular signal-regulated kinase 1/2 (ERK1/2), c-Jun N-terminal kinase/stress-activated protein kinase, and p38 MAPK, is a ubiquitous group of signaling proteins involved in the control of cell growth, differentiation, and adaptation. In skeletal muscles, p38 MAPK activation is necessary for myogenic cell differentiation and is involved in the regulation of glucose metabolism and energy expenditure. Some studies have shown that β-agonists and exercise influence the phosphorylation of p38 MAPK in skeletal muscles. Recently, our group has demonstrated that p38 MAPK phosphorylation is increased, with an occasional increase in p38γ subunit phosphorylation, in slow-twitch muscles after single-dose clenbuterol treatment and acute exercise. However, this effect was not witnessed in fast-twitch muscles. In addition, it has been shown that skeletal muscle contraction, stretch, and overload increases the phosphorylation of p38 MAPK, with an occasional increase in phosphorylated p38γ subunits, to a greater extent in slow-twitch muscles than in fast-twitch muscles.

It has been demonstrated that the p38γ subunit is a key regulator in skeletal muscle metabolic adaptation and muscle fiber type shift, metabolic enzyme activity, and insulin resistance. Thus, β-agonists counteract the beneficial effects of the physiological response to exercise in skeletal muscles.
Fig. 2 The β2-adrenergic (β2-AR) signaling pathway is involved in anabolic and metabolic adaptations to β2-agonist treatment and exercise in skeletal muscles. 

A: The general β2-AR signaling pathway involves the receptor, a heterotrimeric G protein, and the membrane-bound adenylyl cyclase (AC). 

B: The β2-AR signaling pathway involves the agonist- or catecholamine-dependent activation of Gαs, which in turn activates AC, resulting in increased cyclic adenosine monophosphate (cAMP) production. Cyclic AMP-activated protein kinase A (PKA) initiates the transcription of many target genes via phosphorylation of cAMP response element (CRE)-binding protein (CREB) or the adaptor proteins, which subsequently promote protein synthesis. The Gαs-AC-cAMP pathway can also attenuate protein degradation via a Ca2+-dependent pathway. In addition to Gαs, the receptor-coupled Gαi dissociates from the heterodimeric Gβγ, with the free Gβγ subunits mediating the activation of mitogen-activated protein kinase (MAPK) and/or phosphoinositol 3-kinase (PI3K)-Akt/protein kinase B (PKB) pathways. Phosphorylation of Akt is known to have numerous downstream effects. The phosphorylation and subsequent nuclear exclusion of the forkhead transcription factor (Foxo) prevents the transcription of atrophic genes such as MAFbx, MuRF, and genes involved in the inhibition of protein synthesis, such as eukaryotic initiation factor (eIF) 4-binding protein 1 (4EBP-1). Activation of mammalian target of rapamycin (mTOR) increases protein synthesis via the phosphorylation and activation of p70S6K, and the phosphorylation of 4EBP-1 and subsequent activation of eIF-4E. The phosphorylation of MAPK activates cell proliferation and differentiation via direct stimulation of downstream transcription factors such as myocyte enhancer factor 2 (MEF2) and activating transcription factor 2 (ATF2), which initiate the transcription of various genes, such as peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α).
findings suggest that activation of ERK1/2 is necessary for the β₂-agonist-induced hypertrophic process in fast-twitch muscles.

**Effects of β₂-agonists and exercise on phosphoinositol 3-kinase (PI3K)-Akt/protein kinase B (PKB) pathway in skeletal muscles**

Free Gβγ subunits activate the PI3K-Akt signaling pathway (Fig. 2)\(^{11,13,41}\), which has also been implicated in protein synthesis, gene transcription, cell proliferation, and cell survival\(^{42}\). As shown in Table 1, studies in rats have shown that single-dose clenbuterol treatment increases the phosphorylation of Akt in fast-twitch muscles, but not slow-twitch muscles\(^{43,44}\). In contrast to rat models, single-dose administration of clenbuterol in mice increased the phosphorylation of Akt in slow-twitch muscles\(^{44}\). Recently, our group found that Akt phosphorylation in slow-twitch muscles is increased by single-dose clenbuterol treatment in mice, while the fast-twitch muscles were unaffected\(^{22}\). These findings indicate that the effect of β₂-agonists on the phosphorylation of Akt differs between fiber types and species. Moreover, pharmacological inhibition of the Akt signaling pathway revealed that the anabolic response to clenbuterol treatment in rats is greater in fast-twitch muscles than in slow-twitch muscles\(^{45}\). Therefore, activation of Akt in skeletal muscles in response to β₂-AR stimulation may account for the anabolic adaptation.

Table 1 also shows the effects of exercise, overloading, stretch, and electrical stimulation on the phosphorylation and activity of Akt in skeletal muscles. We have recently found that there is no change in the phosphorylation of Akt after acute exercise in skeletal muscles\(^{22}\). Some studies have shown that exercise increases the phosphorylation and activity of Akt in rat skeletal muscles\(^{45,46}\), whereas others have observed no such effect\(^{47}\). Passive stretch and electrical stimulation of rat skeletal muscles was also found to increase the phosphorylation and activity of Akt in fast-twitch muscles, but not in slow-twitch muscles\(^{46-48}\). These findings indicate that the phosphorylation level of Akt differs between experimental models in relation to the intensity of exercise, load, and contraction. However, further experiments are needed to confirm this observation.

**Effects of β₂-agonists and exercise on a current target of β₂-AR signaling, the orphan nuclear receptor, NOR-1, in skeletal muscles**

The NOR-1, known in rats as NR4A3, is thought to be involved in the regulation of genes that control glucose and fatty acid utilization in skeletal muscles. Recently, Pearen et al.\(^{49}\) identified that the promoter region of NOR-1 is a target for β₂-AR-mediated CRE activation in skeletal muscles. An increase in the expression of NOR-1, induced by β₂-AR activation, is consistent with the involvement of PKA, p38 MAPK, and phosphorylated CREB in C2C12 cells\(^{50}\). Additionally, NOR-1 was found to be a negative regulator of myostatin (a member of the transforming growth factor-β superfamily and a potent negative regulator of muscle mass)\(^{50}\). Furthermore, Kawasaki et al.\(^{51}\) demonstrated that acute exercise increases the expression of NOR-1 mRNA in skeletal muscles. These results suggest that β₂-AR activation, through increased NOR-1 expression, inhibits myostatin expression, and thus induces muscle hypertrophy.

**Conclusion**

This review has discussed the effects of β₂-agonists and exercise on β₂-AR signaling in skeletal muscles, which reveals the similarity and difference in the response of β₂-AR signaling molecules between β₂-agonists and exercise. These findings highlight that β₂-AR signaling plays a functional role in anabolic and metabolic adaptations to
β₂-agonists and exercise in skeletal muscles. The insights mentioned in this review will provide scientific evidence for the eradication of β₂-agonists as sports doping agents in terms of furthering our knowledge of the mechanisms of muscle hypertrophy.

Acknowledgments

This work was supported by Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists (2010-2011: S. Sato), and a Grant-in-Aid of the Global Center of Excellence (COE) Program, Graduate School of Sport Sciences, Waseda University (2009-2013: K. Imaizumi) of the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

References


