The potential and the pitfalls of β-adrenoceptor agonists for the management of skeletal muscle wasting

James G. Ryall, Gordon S. Lynch *

Basic and Clinical Myology Laboratory, Department of Physiology, The University of Melbourne, Victoria 3010, Australia

Abstract

The β-adrenergic signaling pathway represents a novel therapeutic target for skeletal muscle wasting and weakness due to its role in the mechanisms controlling protein synthesis and degradation and in modulating fiber type. Stimulation of the pathway with β-adrenoceptor agonists (β-agonists) has therapeutic potential for muscle wasting disorders including: sarcopenia, cancer cachexia, disuse and inactivity, unloading or microgravity, sepsis and other metabolic disorders, denervation, burns, HIV-AIDS, chronic kidney or heart failure, and neuromuscular diseases. However, there are also pitfalls associated with β-agonist administration and clinical applications have so far been limited, largely because of cardiovascular side effects. In rats and mice, newer generation β-agonists (such as formoterol) can elicit an anabolic response in skeletal muscle even at very low doses, with reduced effects on the heart and cardiovascular system compared with older generation β-agonists (such as fenoterol and clenbuterol). However, the potentially deleterious cardiovascular side effects of β-agonists have not been obviated completely and so it is important to refine their development and therapeutic approach in order to overcome these obstacles. This review describes the therapeutic potential of stimulating the β-adrenergic signaling pathway with β-agonists, highlighting the beneficial effects on skeletal muscle structure and function and identifying some of the pitfalls associated with short- and long-term β-agonist administration. The review also identifies some important, but as yet unanswered questions, regarding the importance of β-adrenoceptor signaling in muscle health and disease and the strategies needed to improve the efficacy and safety of β-agonists for muscle wasting disorders.

© 2008 Elsevier Inc. All rights reserved.
1. Introduction

While the primary therapeutic use of β-adrenoceptor agonists (β-agonists) is for bronchodilation in the treatment of asthma, it became apparent that when administered at doses higher than those used therapeutically, these agents could elicit significant skeletal muscle growth. It is this property of β-agonists that has resulted in more than 25 years of research focused on their potential to prevent or reverse the muscle wasting and weakness associated with numerous conditions and pathologies, including sarcopenia (age-related muscle wasting), cancer cachexia, sepsis (and other forms of metabolic stress), denervation, disuse, burns, human immunodeficiency virus (HIV)-acquired immunodeficiency syndrome, chronic kidney or heart failure, chronic obstructive pulmonary disease, muscular dystrophies, and muscular dystrophies and other neuromuscular disorders.

Synthetic β-agonists such as cimaterol, clenbuterol, fenoterol, formoterol, salbutamol and salmeterol, are based on the chemical structure of adrenaline, and promote muscle growth via stimulation of β-adrenoceptors and subsequent activation of downstream signaling pathways. We have recently reviewed the role of β-adrenoceptor signaling in skeletal muscle with implications for health and disease (Lynch & Ryall, 2008). The purpose of this review is not to merely repeat this information, but to focus on the potential (and the pitfalls) of β-agonist therapies for conditions where muscle wasting and weakness are indicated, rather than on β-adrenoceptor signaling per se.

Skeletal muscle contains all three β-adrenoceptor subtypes (β₁, β₂, and β₃-adrenoceptors), with an ~10 fold greater proportion of the β₂-adrenoceptor isoform than either β₁- or β₃-adrenoceptors (Williams et al., 1984; Kim et al., 1991). Although the β₂-adrenoceptors are believed...
to be solely responsible for the β2-agonist-induced skeletal muscle hypertrophy (Hinkle et al., 2002), it is unclear whether β2-adrenoceptors are also responsible for changes to metabolic properties of the muscle. Furthermore, many of the β2-agonists employed in the past have actions on both β1- and β2-adrenoceptors. Therefore, for the remainder of this review, we will refer to the use of β-agonists, rather than discussing specifically the effects of β2-agonists.

### 2. Potential of β-agonist therapy

The hypertrophic response of skeletal (and cardiac) muscle following chronic, high-dose β-agonist administration has been associated with an increase in protein synthesis, a decrease in protein degradation, or a combination of both mechanisms (Lynch & Ryall, 2008). However, results remain equivocal as to the mechanism which predominantly mediates β-agonist-induced growth of skeletal muscle.

Canonical β-agonist signaling has been well described and involves selective coupling to a heterotrimeric G-protein (Gαβγ) to initiate downstream signaling, traditionally believed to occur via the stimulatory Gs subunit (Gαs) coupling to adenylyl cyclase (AC), and resulting in the conversion of ATP to cyclic AMP (cAMP) with subsequent activation of protein kinase A (PKA) (Fig. 1, Lynch & Ryall, 2008). The activation of this pathway has been linked to the inhibition of proteolytic pathways, and possibly to increased protein synthesis.

The Gαβγ subunits of the G-protein have also been suggested to play an active role in various cell signaling processes, which may have important roles in β-agonist-induced hypertrophy of skeletal muscle. Specifically, β-agonists initiate Gαβγ mediated activation of the phosphoinositol 3-kinase (PI3K)-protein kinase B (Akt) signaling pathway, which has been implicated as a regulator of both protein synthesis and protein degradation (Schmidt et al., 2001; Kline, Panaro, Yang, & Bodine, 2007). Thus any discussion of β-agonist mediated hypertrophy must include a clear discussion of their actions on the mechanisms regulating protein synthesis and degradation.

#### 2.1. Inhibition of protein degradation

Increased protein degradation in skeletal muscle is predominantly mediated via four proteolytic pathways: calcium (Ca2+) dependent activation of cytosolic calpains (Costelli et al., 2005), ATP-dependent ubiquitination (Attaix et al., 2005), lysosomal proteases (Farges et al., 2002), and apoptosis (Argilés et al., 2008). The relative contribution of each pathway to muscle breakdown is dependent on the initiating signal (for review see Lynch et al., 2007).

The potential of β-agonists to inhibit protein degradation has been described in numerous animal models (and human conditions) of muscle wasting (Zeman et al., 1987; Carter et al., 1991; Maltin et al., 1993; Agbenyega et al., 1995; Zeman et al., 2000; Kissel et al., 2001; Beitzel et al., 2004; Ryall et al., 2004, 2007, 2008a,b). Although the precise mechanism for the decline in protein degradation remains controversial, it is believed that β-agonists act to inhibit degradation predominantly via the Ca2+-dependent, and ATP/ubiquitin-dependent pathways (Lynch & Ryall, 2008).

#### 2.1.1. Calcium dependent proteolysis

The Ca2+-dependent proteolytic pathway, a major target for β-agonists, is regulated via a family of cysteine proteases called calpains, the most well described being μ-calpain (activated at μM concentrations of Ca2+, also known as calpain I) and m-calpain (activated at mM concentrations of Ca2+, also known as calpain II) (Costelli et al., 2005), and the muscle specific calpain-3 (Sorimachi et al., 1989; Murphy et al., 2007). In skeletal muscle, calpains are localized predominantly in the Z-disk of the sarcomere, and following an initiating signal (such as a rise in intracellular Ca2+), calpains are activated to begin the degeneration of the contractile machinery. Calpain activity is regulated through kinase-mediated phosphorylation (which can both increase and decrease activity depending on the kinase responsible), and the activity of the endogenous calpain-specific inhibitor, calpastatin (Navegantes et al., 2002; Costelli et al., 2005).

Higgins, Lasslett, Bardsley, and Buttery (1988) fed lambs with clenbuterol (2 mg/kg in the feed) for 6 weeks and found a two-fold
increase in the protein concentrations of m-calpain and calpastatin in latissimus dorsi muscles. A follow-up study by Parr, Bardsley, Gilmour, and Buttery (1992) fed Friesian steers with cimaterol (1.5 mg/kg in the feed) for 16 weeks and reported similar increases in m-calpain and calpastatin activity in latissimus dorsi muscles. Similar findings have been observed in cachectic rats and mice treated with formoterol (2 mg/kg, Busquets et al., 2004), and rabbits treated with L644,969 (7 ppm Pringle et al., 1994). The effect of β-agonist administration on m-calpain expression and activity is less clear, with studies showing either no change (Higgins et al., 1988; Parr et al., 1992), or a decrease in activity (Forsberg et al., 1989). These results suggest that β-agonist administration reduces Ca²⁺-dependent proteolysis likely through increased calpastatin activity and possibly that warrant further investigation.

The effect of β-agonist administration on calpain-3 expression and activity has received relatively little attention, and while its function in skeletal muscle has yet to be elucidated clearly, it has been suggested to play an important role in regulating sarcomeric structure (Murphy et al., 2007). Sensky et al. (2006) reported that calpain-3 protein levels were not altered in porcine longissimus muscle 24 h after clenbuterol administration (5 ppm, administered in the feed). Further research is required to determine whether longer treatment periods (and/or a higher dose of β-agonist) are associated with changes in calpain-3 expression/activity.

While β-agonists had been found to alter the protein and mRNA levels of calpain and calpastatin, the first direct measure of the effect of β-agonist administration on Ca²⁺-dependent proteolysis was determined by Navegantes, Resano, Migliorini, and Kettelhut (2001). In this study the authors examined the rate of tyrosine release (an indirect measure of protein degradation) from extensor digitorum longus (EDL) and soleus muscles incubated in the presence or absence of clenbuterol. The rate of Ca²⁺-dependent proteolysis was determined through the use of insulin and branched-chain amino acids (to block lysosomal processes), and the addition of A-23187 (a Ca²⁺-ionophore). These authors reported that clenbuterol inhibited Ca²⁺-dependent proteolysis in EDL and soleus muscles by 30–40%, but did not alter the activities of lysosomal or ATP-dependent proteolysis (Navegantes et al., 2001).

The molecular mechanisms underlying β-agonist-induced inhibition of Ca²⁺-dependent proteolysis have been ascribed to PKA-dependent phosphorylation of numerous proteins, including calpastatin, and possibly calpains and certain sarcoplasmic reticulum (SR) regulatory proteins (Hawkins et al., 1995; Reiken et al., 2003). In addition, the calpastatin gene has been found to contain characteristic cAMP response element (CRE) motifs within the promoter region. Thus, β-agonist-induced activation of PKA would be expected to promote calpastatin transcription through PKA phosphorylation of the CRE binding protein (CREB, Sensky et al., 2006).

2.1.2. Adenosine 5′-triphosphate-dependent proteolysis

In addition to actions on calpain and calpastatin, β-agonists have been associated with changes in ATP-dependent ubiquitination, a process where target proteins are marked for degradation by the 26S proteasome. Ubiquitin is bound first by the ubiquitin activating enzyme (E1) and then transferred to a ubiquitin conjugating enzyme (E2), which acts as a bridge between the non-specific E1 enzyme and the target-specific ubiquitin ligase proteins (E3). The E3 proteins are, in turn, responsible for transferring multiple ubiquitin molecules to the protein targeted for degradation (for review see Attaix et al., 2005; Jin et al., 2007).

One of the earliest studies to examine the role of ubiquitination in the anabolic response to β-agonists was conducted by Costelli et al. (1995). In this study, the authors treated rats inoculated with AH–130 Yoshida ascites hepatoma cells, with clenbuterol (1 mg/kg/day, subcutaneously, s.c.) for 8 days and found a 2–3-fold decrease in the expression of ubiquitin mRNA (Costelli et al., 1995). Later studies confirmed the reports of β-agonist mediated decrease in protein ubiquitination (Busquets et al., 2004; Harcourt et al., 2007; Klíne et al., 2007) and also identified a greater inhibition of ubiquitination in fast- than in slow-twitch muscles (Yimlamai, Dodd, Borst, & Park, 2005).

The activation of the forkhead box O (FOXO) transcriptional program is necessary for the induction of the muscle specific E3 ubiquitin ligase proteins muscle RING finger 1 (muRF1) and muscle atrophy F-box (MAFbx, also called atrogin-1, Stitt et al., 2004), and could therefore play a role in the previously reported decrease in E3 protein expression after β-agonist administration (Fig. 2, Kline et al., 2007). We have recently described this process in detail (see Lynch & Ryall, 2008 for review).

![Fig. 2. β-Agonist mediated inhibition of skeletal muscle proteolysis. Treatment with a β-agonist is thought to inhibit protein breakdown via inhibiting both Ca²⁺-dependent proteolysis, and the FOXO mediated transcription of E3 ubiquitin ligases.](image-url)
MAFbx and mURF have been found to be dramatically increased in a number of muscle wasting conditions (Stitt et al., 2004; Hanai et al., 2007; Lang et al., 2007), and while mURF has been found to target myosin heavy chains (Clarke et al., 2007; Fleitetz et al., 2007), the specific target/s for MAFbx have, until recently, remained a mystery. Lagirand-Cantaloube et al. (2008) demonstrated that one target of MAFbx in skeletal muscle is the eukaryotic initiation factor, eIF3, which plays an important role in the initiation of transcription of muscle structural proteins. The degradation of eIF3 by MAFbx resulted in muscle atrophy, while inhibition of MAFbx by RNAi prevented eIF3 degradation, and subsequent muscle atrophy (Lagirand-Cantaloube et al., 2008). These exciting results demonstrated a previously unidentified potential downstream target for β-agonist administration, but this observation will need to be confirmed in future studies examining β-agonist administration and muscle wasting.

In addition to actions on the process of ubiquitination, a number of studies have indicated that β-agonist administration can reduce the activity of the 26S proteasome (Busquets et al., 2004; Yimlamai et al., 2005). Our current understanding of the mechanisms that regulate the expression and activity of the 26S proteasome in skeletal muscle is severely lacking. Yimlamai et al. (2005) showed that while clenbuterol reduced the activity of the proteasome, the expression levels of the 20S subunit were unchanged. Further research is required to determine the mechanism for the β-agonist-induced decrease in proteasome activity.

2.2. Promotion of protein synthesis

While traditionally examined in relation to proteolytic pathways, β-agonist/AC/cAMP/PKA signaling has been found to initiate transcription of a number of proteins, and thus promote protein synthesis (Berdeaux et al., 2007; Pearen et al., 2006, 2008). Following activation by cAMP, PKA is able to diffuse passively into the nucleus, where it can regulate the expression of many target genes via direct phosphorylation of CREB, or via a modulator that acts on second generation target genes (see Lynch & Ryall, 2008 for review).

In addition to the β-agonist/AC/cAMP/PKA signaling pathway, β-agonist administration activates the PI3K-Akt signaling pathway (Kline et al., 2007). PI3K is believed to phosphorylate the membrane phospholipid phosphatidylinositol-4,5-bisphosphate (PIP2), generating phosphatidylinositol-3,4,5-trisphosphate (PIP3), and initiating the phosphorylation and subsequent activation of the serine/threonine kinase Akt. Akt activation, in turn, initiates numerous signaling pathways involved in protein synthesis, gene transcription, cell proliferation and survival (Glass, 2003; Frost & Lang, 2007).

The β-agonist-induced muscle hypertrophy is associated with phosphorylation of both p70S6K and the eIF4E binding protein 1 (4E-BP1, Sneddon et al., 2001), downstream targets of PI3K/Akt. However, Kline et al. (2007) demonstrated for the first time that β-agonist administration resulted in activation of mammalian target of rapamycin (mTOR) via the PI3K/Akt signaling pathway. In addition, these authors used rapamycin (a pharmacological inhibitor of mTOR) to demonstrate clearly that clenbuterol-induced muscle hypertrophy was differentially regulated in fast- and slow-twitch skeletal muscles (Kline et al., 2007). The differential regulation was evidenced through phosphorylation of both p70S6K and the eIF4E binding protein 1 (4E-BP1, Sneddon et al., 2001), downstream targets of PI3K/Akt. However, Kline et al. (2007) demonstrated for the first time that β-agonist administration resulted in activation of mammalian target of rapamycin (mTOR) via the PI3K/Akt signaling pathway. In addition, these authors used rapamycin (a pharmacological inhibitor of mTOR) to demonstrate clearly that clenbuterol-induced muscle hypertrophy was differentially regulated in fast- and slow-twitch skeletal muscles (Kline et al., 2007). The differential regulation was evidenced through the phosphorylation and subsequent activation of the serine/threonine kinase Akt. Akt activation, in turn, initiates numerous signaling pathways involved in protein synthesis, gene transcription, cell proliferation and survival (Glass, 2003; Frost & Lang, 2007).

The β-agonist-induced muscle hypertrophy is associated with phosphorylation of both p70S6K and the eIF4E binding protein 1 (4E-BP1, Sneddon et al., 2001), downstream targets of PI3K/Akt. However, Kline et al. (2007) demonstrated for the first time that β-agonist administration resulted in activation of mammalian target of rapamycin (mTOR) via the PI3K/Akt signaling pathway. In addition, these authors used rapamycin (a pharmacological inhibitor of mTOR) to demonstrate clearly that clenbuterol-induced muscle hypertrophy was differentially regulated in fast- and slow-twitch skeletal muscles (Kline et al., 2007). The differential regulation was evidenced through the phosphorylation and subsequent activation of the serine/threonine kinase Akt. Akt activation, in turn, initiates numerous signaling pathways involved in protein synthesis, gene transcription, cell proliferation and survival (Glass, 2003; Frost & Lang, 2007).

2.3. Novel myogenic actions of β-agonists

In addition to the traditional hypertrophic actions of β-adrenoceptor stimulation, it is becoming clear that exogenous administration of β-agonists may have numerous novel myogenic actions. β-Adrenergic administration has been found previously to stimulate production of a number of growth factors, including insulin-like growth factors (IGF) and transforming growth factor β (TGFβ) (Sneddon et al., 2001; Akutsu et al., 2006). These growth factors are known to play essential roles in the development, growth, and regeneration of skeletal muscle (Buckingham et al., 2003; Charge & Rudnicki, 2004). Furthermore, the endogenous catecholamines, adrenaline and noradrenaline, have been proposed to play an active role in cell proliferation and differentiation during embryogenesis, suggesting that exogenous β-agonist administration may also alter these processes.

Kim et al. (2008a,b) found that adrenaline administration to mouse embryonic stem cells (eSC) resulted in significant cell proliferation, which was associated with a mitogen-activated protein kinase (MAPK)-dependent increase in the expression levels of the cell cycle proteins cyclin E, cyclin dependent kinase 2 (CDK2), cyclin D1, and cyclin dependent kinase 4 (CDK4). Similarly, C2C12 cells treated with the β-agonist ractopamine exhibited an increased in proliferation, without a discernible change in the rate of differentiation (Shappell et al., 2000). However, Affymetrix gene profiling in mouse skeletal muscle suggests that clenbuterol administration is associated with an increased expression of the CDK inhibitor p21 24 h after administration (Sparlock et al., 2006). An increase in the levels of p21 would be expected to inhibit cell proliferation. Therefore, future experiments using cell culture combined with RNAi inhibition (RNAi), and/or viral directed overexpression of β-adrenoceptors, are required to determine the exact roles of β1-, β2- and β3-adrenoceptors in myoblast proliferation and/or differentiation.

2.4. Alterations in skeletal muscle function

Over the last 25–30 years, there have been numerous studies on animals and several studies on humans examining the effects of β-agonist administration on skeletal muscle function. In most studies on rats and mice, when the animals have been treated chronically with high (mg/kg) doses of β-agonists such as clenbuterol or fenoterol, there is a significant increase in muscle mass and a concomitant increase in absolute force-producing capacity, as determined from force measurements of isolated muscles in vitro or in situ (for a review of many of the published studies pertaining to this topic, see Lynch & Ryall, 2008). These changes are more apparent in fast- than in slow-twitch muscles (Ryall et al., 2002, 2007). However, in some cases only minor (or no) improvement in the force-producing capacity and maximal power output of isolated muscles has been reported following chronic clenbuterol administration (Lynch et al., 2000). In fact, in most cases the force output per muscle cross-sectional area (specific force) is usually unchanged or reduced after chronic β-agonist administration, indicating that the intrinsic force-producing capacity of the muscles does not always keep pace with the obvious increases in muscle mass. The route of administration may also influence the magnitude of the β-agonist-induced changes, and in our hands, systemic administration of rats or mice with a daily intraperitoneal (i.p.) injection of a β-agonist is generally more efficacious (for muscle hypertrophy) than a β-agonist administered via the drinking water (Moore et al., 1994; Ryall et al., 2002).

In humans, β-agonists have been used to increase the muscle tissue mass for cardiac assist surgery where in conjunction with electrical stimulation, a portion of the latissimus dorsi is muscle
regulated after injury, and during aging (Gutmann & Hanzliková, 1966; activity, electrical stimulation, hormonal disturbances, muscle regeneration but they can also occur in response to altered physical circumstances (Schiaffino et al., 2007; Röckl et al., 2008). Other important aspects of muscle function that can be modulated by chronic β-agonist administration include the rates of muscle contraction and relaxation (such as the time to peak twitch response and one-half relaxation time of the twitch response) and muscle fatigue (Schertzer et al., 2005; Ryall et al., 2008a,b). Both of these functional parameters are affected by β-agonist-induced changes in muscle phenotype, typically from slow- to fast-twitch and reflect the changes in muscle fiber composition (see Section 2.5).

2.5. Modulating muscle fiber phenotype

Skeletal muscle fiber types are classified according to their contractile and metabolic properties, including: type I or slow-oxidative, type IIA or fast-oxidative glycolytic, and type IIB or fast-glycolytic, as well as numerous subtypes. For example, within the fast type II fiber category there are three subtypes, IIA, IIB and IId/x (Bar & Pette, 1988; Bortolotto et al., 1999) and there are a multitude of potential hybrid fiber classes based on the existence of numerous isoforms of contractile and regulatory proteins that are possible within individual fibers. The ability of skeletal muscles to undergo constant modification of their morphological and functional characteristics in response to a wide variety of stimuli highlights the plasticity of skeletal muscle. These fiber transformations generally occur during development but they can also occur in response to altered physical activity, electrical stimulation, hormonal disturbances, muscle regeneration after injury, and during aging (Gutmann & Hanzliková, 1966; Houmand et al., 1998; Geiger et al., 2001; Ryall et al., 2007). For example, altering motoneuron firing patterns affects the processes regulating fiber composition (for review see Berchtold et al., 2000) as evident from the transformations observed after intense endurance training, that can increase type I fiber composition under some circumstances (Schiaffino et al., 2007; Röckl et al., 2008). In addition, numerous hormones have been shown to cause alterations in fiber types, including; insulin, thyroid hormone and sex-hormones (Florini et al., 1996; Simonides & van Hardeveld, 2008).

We and others have found that exogenous administration of β-agonists such as clenbuterol, fenotrol and formoterol can result in a dramatic shift in the muscle fiber phenotype from slow-oxidative to faster oxidative-glycolytic fibers (Fig. 3, Zeman et al., 1988; Ryall et al., 2002, 2007). For example, administration of a single intramuscular (i.m.) injection of formoterol (100 μg) directly into the EDL muscles of rats increased the proportion of fast-twitch type IIb fibers by 30% after 2 days (Ryall et al., 2008a). This important observation highlights the rapid rate of fiber remodeling associated with β-agonist administration. While numerous studies have focused on the mechanisms underlying a shift to a slow muscle phenotype (Oh et al., 2005; Handschin et al., 2007; Kim et al., 2008a, b), very little is known about the pathways leading to the fast-twitch phenotype (Grifone et al., 2004; Ryall et al., 2008a).

Studies in rats and mice have shown that a significant shift in slow to fast fiber proportions within skeletal muscles as a consequence of chronic β-agonist administration can dramatically affect function, particularly shortening the duration of the isometric twitch response (Schertzer et al., 2005), increasing velocity of shortening (Dodd et al., 1996), and increasing muscle fatigueability (Dupont-Versteegden, 1996), although these effects are largely dependent on the dose- and β-agonist employed (Harcourt et al., 2007).

2.6. Lipolytic actions and metabolic targets of β-agonists

In addition to the well documented skeletal muscle hypertrophy, β-agonists have been found to alter glucose homeostasis via actions on insulin secretion, liver metabolism, glucose uptake and adipocyte metabolism (Jost et al., 2002; Pan et al., 2001; Erraji-Bencherouk et al., 2005). The combined effects of β-agonist-induced increases in muscle mass and lipolysis have proved desirable for the livestock industry for improving feed efficiency and meat quality, and for enhancing athletic performance (for review see Lynch & Ryall, 2008). β-agonists are thought to stimulate lipolysis through PKA-mediated phosphorylation of hormone-sensitive lipase (HSL) and the lipid-bound protein perilipin A. HSL and/or perilipin A phosphorylation results in an increase in triacylglycerol, diacylglycerol and monoacylglycerol hydrolysis and release of free-fatty acids from the adipocyte (for review see Holm, 2003).
A number of studies have focused on β-adrenoceptor polymorphisms as potential mediators in the development and maintenance of increased adipose stores. Jocken et al. (2007) examined 65 male and 43 female overweight and obese subjects and found a decrease in β1-adrenoceptor mediated lipolysis in subjects with a single-nucleotide polymorphism at codon 16 (Arg16Gly) of the β2-adrenoceptor gene. These results indicate an important role for β2-adrenoceptor signaling in the development or progression of obesity, and suggest a potential therapeutic application for β1-adrenergic in the treatment of obesity and obesity related disorders.

Pearen et al. (2006) reported that β1-adrenergic administration was associated with a significant increase in the expression of the orphan nuclear receptor NOR-1 in skeletal muscle. They determined that the β1-adrenergic formoterol induced a significant increase in NOR-1 expression and, using sRNA techniques, found that NOR-1 targets genes involved in lipid and energy metabolism, including uncoupling proteins (UCP) -2 and -3 (Pearen et al., 2006), which have been linked to mitochondrial uncoupling and lipid metabolism (Fleury et al., 1997; Samec et al., 1999). In a follow-up study, Pearen et al. (2008) found that formoterol treatment transiently increased the expression of genes associated with the oxidation of fatty acids, including peroxisome proliferator-activated receptor γ, coactivator 1α (PGC-1α), lipin1α, FOXO1α and pyruvate dehydrogenase kinase, isozyme 4 (PDK4). These important observations indicated clearly the importance of β1-adrenoceptor signaling in the regulation of oxidative metabolism, following acute stimulation with β1-adrenoceptors.

It is interesting to note that chronic β1-adrenergic administration results in increased glycolytic enzyme activity (phosphofructokinase, PFK; lactate dehydrogenase, LDH) in skeletal muscle, and a concomitant decrease in the oxidative capacity (citrate synthase (CS), cytochrome oxidase (COX), Zeman et al., 1988; Pellegrino et al., 2004). At a functional level, these changes in enzyme activity can result in an increase in muscle fatigability (Ryall et al., 2002). Therefore, it appears that the metabolic effects of β1-adrenergic administration differ following acute or chronic stimulation.

3. Pitfalls of β-adrenergic therapy

While the β-adrenergic signaling pathway represents a novel therapeutic target for age-related muscle wasting and weakness due to its involvement in pathways that modulate skeletal muscle growth and fiber type, it must be recognized that this pathway is highly susceptible to downregulation with chronic stimulation, and this may have detrimental effects once exogenous stimulation is stopped. Also of concern is the presence of β1-adrenoeceptors in tissues other than skeletal muscle. Thus, any approach involving the systemic administration of exogenous β1-adrenergic agonists must take into account effects in tissues other than skeletal muscle, particularly the heart.

Potential side effects associated with long-term therapeutic use of β-adrenoceptor agonists (e.g., for asthma) have been described elsewhere previously (Kendall & Haffner, 1993; Burgess et al., 1997). Similarly, the potential side effects of chronic β1-adrenergic use by athletes for muscle anabolic and lipolytic benefits have been described in detail elsewhere (Lynch, 2002). Excluding athletes or those taking β1-adrenergic for non-medical purposes, there are two groups of individuals exposed to β1-adrenergic; patients being treated with the drugs, and individuals who eat the meat of animals that have been treated with the drugs (Baldi et al., 1994; Sporano et al., 1998). The most frequently reported side effects associated with the use of β1-adrenoeceptors include nausea, headaches and insomnia. Excessive clenbuterol intake can lead to symptoms such as muscle tremor, palpitations, muscle cramps, headache, hypokalemia, hyperglycemia, hypophosphatemia, hyperlactatemia, and peripheral vasodilatation (Prather et al., 1995; Hoffman et al., 2001; Ramos et al., 2004; Schechter et al., 2007; Farr et al., 2008). In many cases, these minor adverse effects are considered predictable and dose-related (Abramson et al., 2003). Athletes stop using β1-adrenoceptors because of headaches, nervousness, insomnia, rapid heartbeat/palpitations, increased blood pressure, nausea, significantly elevated body temperatures, sweating, alternating fevers and chills, nosebleeds, hives, uncontrolled muscle tremor, muscle cramps, or a combination of these factors (Prather et al., 1995). Although any acute effects on motor control such as physiological tremor are relatively asymptomatic in healthy persons (Nizet et al., 2004) and generally not life threatening, they can result in unwanted discomfort and distress. Even so, tolerance levels to β1-adrenergic administration appear highly variable, with one patient trial of albuterol for Duchenne and Becker muscular dystrophy patients reporting no side effects (Fowler et al., 2004) and another reporting only mild tachycardia in a small proportion of patients (Skura et al., 2008). Anecdotal reports advise bodybuilders to take potassium supplements when using clenbuterol in order to control their electrolyte status and reduce muscle cramping (Lynch, 2002). Some bodybuilders using clenbuterol may be especially susceptible to muscle cramps in the time leading up to a contest if they are using diuretics to aid the removal of excess body water, a practice that inevitably could interfere with normal electrolyte balance (Lynch, 2002). Although tachyphylaxis might be considered to be a potential mechanism for many of the adverse side effects of regular β1-adrenergic administration (Abramson et al., 2003), anecdotal reports from bodybuilders and other athletes suggest that the anabolic and/or lipolytic effects of β1-adrenoceptors can be preserved with repeated intermittent treatment rather than continuous administration (Lynch, 2002).

Another concern regarding β1-adrenergic administration is the finding that in animals (rats and mice), appetite can be suppressed for approximately three days after the onset of treatment, which can result in a decrease in body mass (Benson et al., 1991; Choo et al., 1992; Ryall et al., 2007). The loss of appetite in response to acute β1-adrenergic administration may be a consequence of β1-adrenoceptor stimulation of the hypothalamus (Choo et al., 1992), and thus may have implications for individuals whose adipose stores are already low. In these individuals immune competency may be depressed and could be further susceptible to additional complications in the event of reduced nutrition after β1-adrenergic administration (Lesourd & Mazari, 1999).

3.1. Skeletal muscle tremors and increased fatigability

Of functional and clinical significance is whether β1-adrenergic administration affects muscle fatigue resistance deleteriously. Thus, a β1-adrenergic-induced shift from slow (oxidative) to fast (glycolytic) metabolism could be associated with increased muscle fatigue and so reduce the clinical merit of any proposed intervention for muscle wasting or related neuromuscular disorders. When β1-adrenergic treatment has been associated with slow-to-fast fiber transitions in rat or mouse muscles, an increased susceptibility to muscle fatigue has been reported (Zeman et al., 1988; Bricout et al., 2004; Ryall et al., 2004; Baker et al., 2006). However, it should be noted that the effects on fatigue resistance are also likely dependent on the agonist and especially the dose employed. Treatment of dystrophic mdx mice with formoterol (25 μg/kg/day i.p. for 4 weeks) was sufficient to elicit muscle hypertrophy, but was not associated with changes in muscle fatigue resistance (Harcourt et al., 2007). This is important since muscles from boys with Duchenne muscular dystrophy are already highly susceptible to fatigue (Sockolov et al., 1977) and so any intervention that alters muscle metabolism to such an extent that fatigability is affected would be considered undesirable.

3.2. Potential deleterious cardiovascular effects

Perhaps the most serious side effects of excessive β1-adrenergic intake are those associated with the heart and cardiovascular system (Au et al., 2000; Deligiannis et al., 2006). Since the heart and blood vessels contain a large population of β1-adrenergic, any treatment...
strategy involving systemic administration of β-agonists must take into account potential effects on the cardiovascular system. For example, the use of β-agonists has been reported to increase risk for ischemia, congestive heart failure, arrhythmias and sudden death (Fisher et al., 2004; Deligiannis et al., 2006).

Unlike skeletal muscle where the β2-adrenoceptor is the predominant subtype, cardiac muscle contains approximately 70% β1-adrenoceptors, and 30% β2-adrenoceptors (in both rodents and humans, Ryall et al., 2002), with both subtypes involved in regulatory mechanisms of cardiac function (van der Heyden et al., 2005; Xiao et al., 2006). Recent work examining the function of these two receptors in the heart has revealed several striking physiological differences between β1- and β2-adrenoceptor signaling (Xiao et al., 1995; 1999). Despite the dominant role for the β-adrenoceptor–Gαi–cAMP pathway, cardiac β2-adrenoceptors are capable of coupling to the pertussis toxin (PTX) sensitive Gqi protein (Xiao et al., 1995). Thus, activation of Gqi may deliver a Gqi-independent signal (Xiao et al., 1994), through the activation of the MAPK cascade, also known as extracellular signal-regulated kinase 1 (ERK1, Schmitt & Stork, 2000; Sellers et al., 2000; Wetzker & Bohmer, 2003). Regardless of the underlying mechanism, systemic β-adrenoceptor administration can have a number of significant effects on cardiac function.

The acute effects of β-agonist administration on cardiovascular function measured in vivo and/or in vitro have revealed a number of important changes in cardiovascular performance, including an increased force of contraction (positive inotropic response, Molenar et al., 2007), an increased rate of relaxation (positive lusitropic response, Kentish et al., 2001), a decrease in BP, and an increase in heart rate (HR) (positive chronotropic response, Ryall et al., 2008b) as a consequence of one or more of these effects or perhaps directly through β-adrenergic stimulation.

The most well documented effect of chronic β-agonist administration (at least in animal models) is cardiac hypertrophy (Carbo et al., 1997; Sneddon et al., 2001; Ryall et al., 2002; Busquets et al., 2004; Ryall et al., 2004, 2006). However, there remains some controversy as to whether this β-agonist-induced cardiac hypertrophy is a physiological or a pathological response, and may be dependent on the condition of the heart prior to initiating treatment (Wong et al., 1998; Soppa et al., 2005; Ryall et al., 2008b). Similarly, administration of the β-agonist isoproterenol induced ventricular arrhythmias in rabbits suffering from heart failure but not control rabbits (Pogwizd et al., 2001). These arrhythmogenic effects of β-agonist infusion have been linked to an increase in SR Ca2+ load and the consequent spontaneous SR Ca2+ release (DeSantiago et al., 2008).

Histological examination of the myocardium of dogs after chronic treatment with isoprenaline at high (mg/kg) doses revealed severe necrosis (Kendall & Haffner, 1993). Congestion, interstitial edema, hypertyrophi of cardiomyocytes and myocardial necrosis were evident in rats administered very large doses (between 17 and 150 mg/kg daily) of another β2-agonist, salbutamol, for one month (Libretto 1994), with increases in cardiac mass of up to 27% reported (Yamada et al., 1977). Severe myocardial lesions were found in the hearts of sheep given intravenous doses of either salbutamol, fenoterol, or isoprenaline (128 μg/kg at 15 minute intervals), for four days (Pack et al., 1994). Isoproterenol treatment produced necrosis and increased collagen infiltration in the hearts of rats (Benznak, 1962) even when applied in low doses (Benjamin et al., 1989).

Shorter periods of clenbuterol administration to rats did not affect cardiac function despite a 26% increase in left ventricular hypertrophy (Wong et al., 1998). Similar findings of little or no change in cardiac function have been reported in rats treated with low doses (0.2–0.4 mg/kg body mass) of isoproterenol (Baldwin et al., 1982; Taylor & Tang, 1984). The isoproterenol-induced cardiac hypertrophy was not homogenous among the rats and cardiac mass, cardiac relaxation, left ventricular stiffness and systolic function differ between subgroups of animals (Murd & Tucci, 2000).

Conversely, rats administered high doses of clenbuterol (2 mg/kg) daily for several months exhibited cardiac hypertrophy and collagen infiltration in the left ventricular wall (Duncan et al., 2000) and the cardiac hypertrophy in adult rats following fenoterol treatment (1.4 mg/kg i.p. daily) was associated with an increase in left ventricular developed pressure, a marked reduction in cardiac output, and a reduction in coronary flow per unit heart mass (Gregorevic et al., 2005). Clearly, the dosages of β-agonists employed in these studies were higher than those used by athletes. However, it should also be recognized that the doses of β-agonists employed by bodybuilders would typically exceed that recommended for therapeutic purposes. The adverse effects of very high-dose older generation β-agonists (like clenbuterol and fenoterol) on the heart highlight the side effects and dangers associated with the abuse of these drugs. In addition, bodybuilders often use clenbuterol in conjunction with anabolic steroids, a combination that can be lethal (Goldstein et al., 1998; Kierzkowska et al., 2005).

3.2.1. β-Adrenergic administration to healthy individuals

Some early preclinical and clinical observations implicated β-agonists in the development of hypertrophic myocardial pathology (Jeppsson et al., 1988; Benjamin et al., 1989). Furthermore, chronic use of β-agonists for the prevention and relief of asthma symptoms has been associated with an increased risk for adverse cardiovascular related events (Salpeter et al., 2004).

We have previously shown that chronic administration of the β1-agonist fenoterol (1.4 mg/kg/day, i.p.) to rats is associated with an increase in left ventricular developed pressure, a reduction in cardiac output and coronary flow, and a prolongation of diastolic relaxation (Gregorevic et al., 2005). Of particular concern was the finding of a β1-agonist-induced prolongation of cardiac relaxation. During times of rapid heart rate, the duration of diastole is shortened to a greater extent than systole and since most ventricular filling occurs early in diastole, any prolongation of diastolic relaxation could severely impair cardiac filling during times of stress, such as intense exercise (Fioretti et al., 1980).

In a follow-up study, we used implantable radio-telemeters to examine the cardiovascular response of rats to 4 weeks of formoterol treatment (25 μg/kg/day, i.p.). Chronic formoterol administration was associated with an initial increase in HR, a decrease in mean arterial blood pressure (MAP), and an increase in the rate of relaxation. Interestingly, once formoterol treatment was withdrawn, HR decreased, MAP was restored to pre-treatment levels, and similar to our previous findings, there was a prolongation of diastolic relaxation (Ryall et al., 2008b). We also examined the potential effects of formoterol on the cardiac SR Ca2+-ATPase (SERCA), the major determinant of cytosolic [Ca2+] (Bers, 2002), and found that the formoterol-induced prolongation of diastolic relaxation was associated with a 30% decrease in the maximal SERCA2a activity (Ryall et al., 2008b).

In contrast to the detrimental cardiovascular effects of formoterol treatment (Ryall et al., 2008b), chronic administration of the β1-agonist clenbuterol (2 mg/kg/day, s.c.) to rats has been suggested to result in cardiac hypertrophy with normal functional, morphological, and molecular properties (Wong et al., 1998). A direct comparison of the cellular and molecular responses to formoterol or clenbuterol treatment may help elucidate important differences in the downstream signaling pathways activated by these agonists.

3.2.2. β-Adrenergic administration under pathological conditions

Our recent findings indicating that chronic β1-agonist stimulation is detrimental to cardiac function are in contrast to previous studies in animal models of heart failure, where chronic β1-agonist stimulation improved cardiac function in dilated cardiomyopathy (DCM) (Ahmet et al., 2004, 2005, 2008). These results suggest that chronic β1-agonist administration can have either beneficial or detrimental effects on cardiac function depending on the pre-treatment condition of the heart.
In animal models of chronic heart failure a decrease in β₁-adrenoceptor density and an uncoupling of β₂-adrenoceptors from the Goₛ signaling pathway is evident (for review see Lohse et al., 2003; Feldman et al., 2008). Therefore, under these conditions, the administration of a β-agonist may elicit an improvement in cardiac function. Furthermore, seminal work by Xiao et al. showed that activation of the β₂-adrenoceptor (via an inhibitory Goₛ dependent mechanism) signals to promote cell survival, which may attenuate cell death and prevent the progression of a number of cardiac pathologies (Xiao et al., 1999a,b). However, the continued exposure of β-adrenoceptors to an agonist results in a rapid attenuation of receptor responsiveness (desensitization), and if the agonist is not removed, a decrease in β-adrenoceptor number (downregulation, Lynch & Ryall, 2008), which could likely impair cardiac function. Therefore, if β-agonists are to be employed in their current form to treat muscle wasting and weakness, these discrepancies in the cardiac response to these drugs must be clarified.

4. Future directions for therapeutic approaches utilizing β-agonists

As described in Section 3, some of the most serious consequences associated with chronic β-agonist administration relate to the systemic responses to β-adrenoceptor activation. Much research is currently focused on developing new methods of drug administration that limit unwanted systemic effects, with many having the potential to improve the safe delivery of β-agonists to skeletal muscle.

Many muscle wasting conditions, such as that associated with the normal process of aging, require only small (10–15%) increases in muscle mass and strength to significantly improve the quality of life. We have shown previously that the β-agonist formoterol is capable of eliciting significant skeletal muscle hypertrophy at doses as low as 1 μg/kg/day in rats (Ryall et al., 2004). In a subsequent study we found that even a dose as low as 25 μg/kg/day was sufficient to elicit a significant increase in the maximal force-generating capacity of skeletal muscles from old mice. Importantly, we showed that at this dose formoterol treatment was not associated with any measurable level of cardiac hypertrophy in old rats (Ryall et al., 2007). These results demonstrate that the β-agonist-induced beneficial skeletal muscle hypertrophy, and potentially deleterious cardiac hypertrophy, can be separated on the basis of dose.

Following the discovery of long-acting β-agonists such as formoterol and salmeterol, much research has focussed on extending the actions of β-agonists even further (a new class of agents termed ultra long-acting β-agonists, Matera & Cazzola, 2007). A number of new agents, including arformoterol, carmoterol, indacaterol and GSK-159797 are currently in phase II–III clinical trials for the treatment of asthma and chronic obstructive pulmonary disorder (Matera & Cazzola, 2007). It will be interesting to see what effect these new agents have on skeletal muscle, and whether these agents have an increased safety profile over existing β-agonists.

4.1. Localization of treatment

We have previously examined whether direct i.m. injection of the β-agonist formoterol can localize its effects to skeletal muscle directly and so minimize any potential detrimental systemic effects (Ryall et al., 2008a). Two days after a single i.m. injection of formoterol, the force-producing capacity of regenerating rat EDL muscles was two-fold higher than that of regenerating EDL muscles that received a single i.m. injection of saline. Importantly, i.m. administration of formoterol was not associated with cardiac hypertrophy. However, it should be noted that the increase in muscle mass and force-producing capacity after i.m. administration was lost within 5 days, and was still associated with a number of changes in cardiovascular function, including a transient increase in HR and a decrease in BP.

4.2. Co-administration of a β₂-agonist with a β₁-agonist

Blocking stimulation of the β₁-adrenoceptors is possible with highly selective β₁-adrenoceptor antagonists such as CGP 20712A (Silence & Matthews, 1994) and the importance of blocking β₁-adrenoceptors in heart failure to abrogate cardiotoxic β₁-adrenoceptor-mediated effects is also well known (Molenaar & Parsonage, 2005; Ahmet et al., 2008). The fact that formoterol is highly selective for the β₂-adrenoceptor compared with older generation agonists such as albuterol and clenbuterol (Anderson, 1993), and that it is efficacious in eliciting skeletal muscle anabolic effects even at micromolar doses (Ryall et al., 2006), offers the considerable advantage that simultaneous β₁-adrenoceptor blockade may prevent or attenuate many of these cardiovascular side effects. In a recent commentary, Molenaar, Chen, and Parsonage (2006) suggested that the use of highly selective β₂-agonists, in conjunction with a selective β₁-blocker, could prevent unintended β₁-adrenoceptor activation and thus prevent unwanted cardiovascular effects while maintaining desirable effects on the skeletal muscle. This is particularly important for cardiovascular β₁-adrenoceptors, where chronic activation of β₁-adrenoceptors is contraindicated for prevalent cardiac and vascular disorders including hypertension, ischemic heart disease, arrhythmias and heart failure where β-blockers are indicated. A pathological role of the β₁-adrenoceptor was confirmed in transgenic mice where 15-fold overexpression led to progressive deterioration of heart function, hypertrophy and heart failure (Engelhardt et al., 1999).

Thus, future studies examining dose-optimization of current and newly synthesized β-agonists and studies investigating the potential for selective β₁-adrenoceptor blockade to prevent unwanted cardiovascular effects may yield important information regarding the therapeutic application of these agents for the successful treatment of skeletal muscle wasting and weakness (Lynch et al., 2008).

4.3. Novel therapeutic targets

4.3.1. Goₛ vs. Goₚ signaling

Studies examining the difference between β₁- and β₂-adrenoceptor-mediated signaling in the heart have revealed dichotomous G-protein coupling in β₂-adrenoceptors (described in Section 3.2). Unlike the β₁-adrenoceptor which couples exclusively to the Goₛ protein, the β₂-adrenoceptor couples both the stimulatory Goₛ and the inhibitory Goₛ proteins to elicit downstream signals (Murga et al., 2000; Xiao, 2001; Cosmanov et al., 2002). Xiao et al. showed that disruption of Goₛ signaling by PTK enhanced the β₂-adrenoceptor-induced contractile response in rat and mouse ventricular myocytes (Xiao et al., 1995, 1999; Xiao, 2000). Phosphorylation labeling of G-proteins with [³²P]-azonoiilido-GTP in conjunction with immunoprecipitation with antibodies specific for Goₛ and Goₚ has provided direct biochemical evidence that cardiac β₂-adrenoceptors activate both Goₛ and Goₚ signaling pathways, while the β₁-adrenoceptor selectively activates Goₛ (Kilts et al., 2000). Numerous studies have implicated PKA in the regulation of β₂-adrenoceptor signaling, where PKA-mediated phosphorylation appears to enhance the interaction of the β₂-adrenoceptor with Goₛ (Daaka et al., 1997; Okamoto et al., 1991). This finding for dual coupling has raised issues regarding the physiological and pathophysiological relevance of coupling of Goₛ in addition to Goₛ (2000; Xiao et al., 1999a,b).

Recent evidence suggests that stimulation of both β₁- and β₂-adrenoceptors results in a cAMP-dependent increase in myocyte apoptosis (Communal et al., 1999; Pönöcke et al., 2003). However, selective stimulation of the β₂-adrenoceptor inhibits apoptosis via a Goₛ-coupled pathway (Communal et al., 1999; Foerster et al., 2003; Pönöcke et al., 2003). Thus, the β₂-adrenoceptor appears to oppose the proapoptotic signal generated via the β₁-adrenoceptor–Goₛ–PKA–cAMP signaling pathway.

The different roles of β₁-adrenoceptor–Goₛ/Goₚ coupling in skeletal muscle have so far received only limited attention. Beitzel, Silence,
and Lynch (2007) found that similar to β-adrenoceptor density, rat slow-twitch soleus muscles expressed an –two-fold greater density of Goα, compared with fast-twitch muscles. As described in Section 2.5, β-adrenergic induces muscle hypertrophy appears to be differentially regulated in fast- and slow-twitch skeletal muscles, and we are currently examining β-adrenoceptor–Goα, coupling as a potential mechanism to explain this difference.

4.3.2. Phosphodiesterase inhibitors

Phosphodiesterase (PDE) is the enzyme responsible for the degradation of cAMP into 5′-AMP, and it therefore plays an important role in terminating the PKA–cAMP signaling cascade (for review see Omori & Kotera, 2007). Skeletal muscle contains numerous isoforms of PDE, including: PDE4, PDE7, and PDE8, however, PDE4 is believed to be predominantly responsible for cAMP degradation in this tissue (Bloom, 2002).

Selective inhibitors of PDE have been used to treat a diverse range of pathological conditions, including chronic obstructive pulmonary disorder, erectile dysfunction, and hypertension (Benedict et al., 2007; Kass et al., 2007; Burnett, 2008). However, the potential of PDE inhibitors to treat skeletal muscle wasting and weakness has received only limited attention. Some of the earliest studies in skeletal muscle utilized the non-selective PDE inhibitor pentoxifylline. Hudlická and Price (1990) found that 5 weeks of tri-daily administration of pentoxifylline (3 mg/kg, i.p.) to rats increased the proportion of glycolytic fibers in EDL muscles. Breuillé et al. (1993) demonstrated that a single injection of pentoxifylline (100 mg/kg, i.p.) to rats was sufficient to significantly attenuate the atrophy of the gastrocnemius muscle associated with 6 days of induced sepsis. Hindké, Dolan, Cody, Bauer, and Isfort (2005) administered either rolipram or Ariflo (both selective PDE4 inhibitors) or pentoxifylline via twice-daily s.c. injections to rats and mice after denervation or during disuse atrophy (limb-casting), respectively. PDE4 selective or PDE non-selective inhibition had little or no effect on muscle mass and strength in control muscles, while all three pharmacological inhibitors prevented the loss of muscle mass associated with denervation or disuse by ~20–40%. The results from these studies suggested a role for PDEs in proteolytic processes, and this was confirmed by Baviera, Zanon, Carvalho Navegantes, Migliorini, and do Carmo Kettelhut (2007) who showed that pentoxifylline administration to diabetic rats reduced the activity of the Ca2+-dependent and ATP proteasome-dependent proteolytic pathways.

An attractive hypothesis is that selective PDE inhibitors may be sufficient to prevent, attenuate, or reverse muscle wasting and weakness, without the complicating cardiac side effects associated with β-adrenergic administration. However, it must be noted that chronic administration of the non-selective PDE pentoxifylline is associated with a rightward shift of the left ventricular end-diastolic pressure–volume relationship, thinning of the left ventricular wall, and increments in myocardial collagen (Anamourlis et al., 2006).

4.3.3. Modulation of A kinase anchoring proteins

One of the primary mechanisms responsible for the localization of cAMP signaling to specific substrates involves scaffolding proteins, such as A kinase anchoring proteins (AKAPs), which bind numerous cAMP effector molecules including PKA, PDEs, ERK, and protein phosphatases, such as calcineurin (Michel & Scott, 2002; Lynch & Ryall, 2008). Following cAMP activation, PKA linked to AKAP initiates a response that is localized to the AKAP targeted region. Therefore, any disruption to the AKAP molecule would likely have a dramatic effect on cAMP/PKA signaling.

The muscle specific AKAP (mAKAP) has been found to be involved in tethering PKA to the SR, where it facilitates PKA-mediated phosphorylation of the ryanodine receptor (RyR) and subsequent Ca2+–release, resulting in both contractile responses and activation of Ca2+-dependent signaling pathways (Ruehr et al., 2003). While numerous studies have examined the role of mAKAP in the development of cardiac hypertrophy (for review see Bauman et al., 2007), the role of this important scaffold protein has yet to be examined in skeletal muscle in detail. For example, Pare et al. (2005) reported that mAKAP was important for β-adrenergic induced cardiac hypertrophy, since disruption of mAKAP function via siRNA prevented cardiac myocyte hypertrophy in a calcineurin/nuclear factor of activated T-cells (NF-AT)-dependent manner. Clearly, the role of mAKAP in β-adrenerg-induced skeletal muscle hypertrophy warrants further investigation.

Reynolds, McCalmon, Tomczyk, and Naya (2007) have identified a novel AKAP, termed myospryn, that is localized to the Z-disc/costameric region in skeletal muscle. In skeletal muscles of dystrophic mdx mice, PKA activity was decreased, mediated in part by a mislocalization of myospryn (Reynolds et al., 2008). Further research is required to determine the targets of PKA (and binding partners for myospryn) in the costamere.

4.3.4. Selective activation, or inhibition of β-arrеstins

β-Arrestin belongs to a family of proteins responsible for arresting β-adrenoceptor/G-protein-dependent signaling and mediating receptor endocytosis. The arrestin protein family in mammals includes arrestin1 (visual or rod arrestins), arrestin2 (also termed β-arrestin), arrestin3 (β-arrestin2) and arrestin4 (cone-arrestin or X-arrestin) (Gurevich & Gurevich, 2006). While the classical role of β-arrestins in β-adrenergic desensitization, internalization, and downregulation, has been reviewed extensively (Luttrell & Lefkowitz, 2002; Lynch & Ryall, 2008), it is the more recently identified role of β-arrestins as transducers of cell signaling that is of importance to the current discussion.

Daaka et al. (1998) used HEK293 cells and dominant negative mutants to demonstrate an essential role of β-arrestin in the β-adrenergic-mediated activation of the MAPK signaling pathway. Since this seminal discovery, β-arrestins have been found to activate numerous downstream pathways, including mitogenic signaling through ERK1/2, activation of the small GTPase RhoA, as well as inhibition of nuclear factor-kappa B (NF-kB) signaling (see DeWire et al., 2007 for review).

These studies suggest that in addition to the canonical β-adrenoceptor/G-protein signaling pathway, there is a G-protein independent signaling pathway, likely mediated via β-arrestin. The development of novel pharmaceutical agents that can selectively activate the β-arrestin signaling pathway will greatly aid in our understanding of this novel pathway.

5. Summary and conclusions

The β-adrenergic signaling pathway represents a novel therapeutic target for the treatment of skeletal muscle wasting and weakness due to its critical roles in the mechanisms controlling protein synthesis and degradation and the modulation of muscle fiber type (Lynch & Ryall, 2008). Although stimulation of the β-adrenergic signaling pathway with β-agonists has great therapeutic potential for muscle wasting disorders, there are some obvious pitfalls with this approach and clinical applications have so far been limited, largely because of cardiovascular side effects. Newer generation β-agonists (such as formoterol) can elicit an anabolic response in skeletal muscle even at very low doses, with reduced effects on the heart and cardiovascular system compared with older generation β-agonists (such as fenoterol and clenbuterol). However, the potentially deleterious cardiovascular side effects of β-agonists have not been obviated completely and so it is important to continue to refine their development and therapeutic approach in order to overcome these obstacles.

As our knowledge regarding the importance of β-adrenoceptor signaling in skeletal muscle health and disease increases, it is apparent that a number of significant health-related issues remain unanswered. Do older asthmatics that have been receiving...
chronically for a large portion of their lives exhibit less muscle wasting and weakness compared with otherwise healthy older men and women? Do older patients taking β-antagonists exhibit a greater or lesser degree of sarcopenia? Polymorphisms in different receptors are associated with muscle strength in men and women, including the vitamin D receptor gene (Windeløck et al., 2007), glucocorticoid receptor (Peeters et al., 2008) and the CNF and CNF receptor genes (Roth et al., 2003; De Mars et al., 2007). What about polymorphisms in the β-adrenergic receptor gene? Previous studies have suggested that genetic polymorphisms that influence the balance between beneficial and toxic effects of β-adrenergic receptor signaling may be important to the outcomes of cardiac disease (Liggett et al., 2008) and cardiopulmonary responses to exercise (Snyder et al., 2008). This might be especially relevant to the use of β-adrenergic receptor blockade for treating cardiac failure and ischemia and when considering how genetic variants of G-protein-coupled receptor (GPCR) kinases (GRKs) can desensitize β-adrenergic receptor signaling in the heart (Liggett et al., 2008). Is there a similar association of β-adrenergic subtypes with muscle size and strength? There is a dearth of knowledge regarding these important health questions that have obvious implications for the maintenance of skeletal muscle size, function, and quality of life. A better understanding of the role of β-adrenergic receptor signaling in skeletal muscle will help identify more effective β-agonist treatment strategies for muscle wasting disorders.

Acknowledgments

The funding for this research has been provided by generous grants from the Australian Research Council Discovery-Project funding scheme (DP0665071, DP0772781), the National Health and Medical Research Council of Australia (350439, 454561, 509313), the Muscular Dystrophy Campaign of Australia (DP0665071, DP0772781), the National Health and Medical Research Council of Canada (350439, 454561, 509313), the Muscular Dystrophy Campaign of Australia (350439, 454561, 509313), the Muscular Dystrophy Campaign of Australia (350439, 454561, 509313), and the Muscular Dystrophy Campaign of Australia (350439, 454561, 509313).

References

cardiopulmonary position paper on adverse cardiovascular effects of doping in athletes. 


