

Role of β -Adrenoceptor Signaling in Skeletal Muscle: Implications for Muscle Wasting and Disease

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I. Introduction	730
II. Adrenoceptors and the Sympathetic Nervous System	730
A. The guanine nucleotide-binding regulatory proteins	731
B. The α -adrenoceptors	733
C. The β -adrenoceptors	733
III. β -Adrenoceptor Signaling in Skeletal Muscle	733
A. Skeletal muscle β -adrenoceptor subtypes	733
B. G protein coupling in skeletal muscle	734
C. cAMP	734
D. Downstream effectors of cAMP	735
E. Localization of cAMP signaling: A kinase anchoring proteins	737
F. PKA regulation of intracellular calcium signaling	737
G. PKA-mediated skeletal muscle growth and development	738
H. PKA independent signaling pathways in skeletal muscle	739
I. β -Adrenergic-mediated apoptosis	740
J. Regulation of β -adrenoceptor expression and function	740
IV. β -Adrenoceptor Agonists	743
A. Acute response to β -adrenoceptor agonist stimulation	744
B. Effects of chronic β -adrenoceptor agonist administration: evidence from animal studies	745
V. Therapeutic Potential of β -Agonists: Implications for Muscle-Wasting Disorders	747
A. Age-related muscle wasting and weakness	747
B. Muscular dystrophy	750
C. Motor neuron disease: amyotrophic lateral sclerosis	751
D. Denervation and muscle unloading	751
E. Catabolic stresses: burns, sepsis, cancer cachexia, and COPD	752
F. Improving muscle regeneration and functional repair after injury	752
VI. Potential Limitations of β -Agonist Therapy and Implications for Their Abuse	753
A. Effects of β -agonists on cardiac muscle structure and function	753
B. Use of β -agonists by athletes for performance enhancement	754
VII. Concluding Remarks	755

Lynch GS, Ryall JG. Role of β -Adrenoceptor Signaling in Skeletal Muscle: Implications for Muscle Wasting and Disease. *Physiol Rev* 88: 729–767, 2008; doi:10.1152/physrev.00028.2007.—The importance of β -adrenergic signaling in the heart has been well documented, but it is only more recently that we have begun to understand the importance of this signaling pathway in skeletal muscle. There is considerable evidence regarding the stimulation of the β -adrenergic system with β -adrenoceptor agonists (β -agonists). Although traditionally used for treating bronchospasm, it became apparent that some β -agonists could increase skeletal muscle mass and decrease body fat. These so-called “repartitioning effects” proved desirable for the livestock industry trying to improve feed efficiency and meat quality. Studying β -agonist effects on skeletal muscle has identified potential therapeutic applications for muscle wasting conditions such as sarcopenia, cancer cachexia, denervation, and neuromuscular diseases, aiming to attenuate (or potentially reverse) the muscle wasting and associated muscle weakness, and to enhance muscle growth and repair after injury. Some undesirable cardiovascular side effects of β -agonists have so far limited their therapeutic potential. This review describes the physiological significance of β -adrenergic signaling in skeletal muscle and examines the effects of β -agonists on skeletal muscle structure and function. In addition, we examine the proposed beneficial effects of β -agonist administration on skeletal muscle along with some of the less desirable

cardiovascular effects. Understanding β -adrenergic signaling in skeletal muscle is important for identifying new therapeutic targets and identifying novel approaches to attenuate the muscle wasting concomitant with many diseases.

I. INTRODUCTION

While the importance of β -adrenergic signaling in the heart has been well documented for more than half a century and continues to receive significant attention, it is only more recently that we have begun to understand the importance of this signaling pathway in skeletal muscle. There is considerable evidence regarding the stimulation of the β -adrenergic system with β -adrenoceptor agonists (β -agonists) in animals and humans. Although traditionally used for the treatment of bronchospasm, it became apparent that some β -agonists had the ability to increase skeletal muscle mass and decrease body fat. These so-called "repartitioning effects" proved desirable for those working in the livestock industry trying to improve feed efficiency and meat quality. Not surprisingly, β -agonists were soon being used by those engaged in competitive bodybuilding and by other athletes, especially those in strength- and power-related sports.

As a consequence of their muscle anabolic actions, the effects of β -agonist administration on skeletal muscle have been examined in a number of animal models (and in humans) in the hope of discovering therapeutic applications, particularly for muscle wasting conditions such as sarcopenia (age-related muscle wasting and associated weakness), cancer cachexia, sepsis, and other forms of metabolic stress, denervation, disuse, inactivity, unloading or microgravity, burns, human immunodeficiency virus (HIV)-acquired immunodeficiency syndrome, chronic kidney or heart failure, chronic obstructive pulmonary disease, muscular dystrophies, and other neuromuscular disorders. For many of these conditions, the anabolic properties of β -agonists may attenuate (or potentially reverse) the muscle wasting, muscle fiber atrophy, and associated muscle weakness. β -Agonists also have clinical significance for enhancing muscle repair and restoring muscle function after injury or following reconstructive surgery.

Despite their muscle anabolic properties, β -agonists have also been associated with some undesirable side effects, including increased heart rate (tachycardia) and muscle tremor, which have so far limited their therapeutic potential. In fact, many athletes are not aware of the deleterious cardiovascular effects of chronic high-dose β -agonist administration and in many cases rely on anecdotal information about these compounds from nonscientific sources. The purpose of this review is to describe the physiological significance of β -adrenergic signaling in skeletal muscle. We also review the effects of β -adrenergic stimulation using β -agonists and their effects on skeletal muscle structure and function, as well as their mechanism of action. We describe

the use of β -agonists by athletes for the purpose of enhancing sporting performance and body appearance and provide a balanced account of the proposed beneficial effects of β -agonist administration on skeletal muscle along with some of the less well-reported deleterious effects of chronic β -agonist administration on cardiovascular parameters and exercise performance. A greater understanding of β -adrenergic signaling in skeletal muscle is important for identifying its role in muscle growth, development, and muscle regeneration and for identifying new therapeutic targets. Research is needed to understand how the β -adrenergic signaling pathway can be manipulated for the purposes of 1) attenuating the muscle wasting associated with many diseases and conditions and 2) enhancing muscle fiber growth and improving the functional repair of damaged and regenerating skeletal muscle after injury.

II. ADRENOCEPTORS AND THE SYMPATHETIC NERVOUS SYSTEM

The sympathetic nervous system is comprised of two major chemical signaling molecules, the catecholamines adrenaline (epinephrine) and noradrenaline (norepinephrine). Adrenaline is produced and released from the adrenal glands, and noradrenaline is produced and released from nerve axons following stimulation with acetylcholine. Binding of one of these chemicals to an adrenergic receptor will elicit a response, depending on the receptor subtype bound.

In 1948 these adrenergic receptors, termed "adrenoceptors," were divided into two major subgroups. Dr. Raymond Ahlquist (8) published a now classic study on the effects of six different sympathetic stimulating drugs on a variety of adrenergic responses, mostly related to their effects on cardiac muscle. He designated the two groups of agonists: alpha (α), which elicited an excitatory response, and beta (β), which resulted in mostly inhibitory responses (8). We now know that the adrenergic system is more complex than this, but Ahlquist's early delineation of the subtypes remains. To date, there are at least nine subtypes of adrenoceptors that have been cloned, including six α - and three β -subtypes, which are located in different proportions in numerous tissues throughout the body (Table 1), with the β -adrenoceptor family predominating in skeletal muscle.

Over the past 20 years there have been a multitude of studies demonstrating the growth-promoting actions of β -adrenoceptor stimulation in skeletal muscle (5, 6, 31, 74,

TABLE 1. *Adrenoceptor subtype and their respective G proteins and effectors*

Adrenoceptor:	α		β			
	Isoforms:	α_{1A} , α_{1B} , and α_{1D}	α_{2A} , α_{2B} , and α_{2C}	β_1	β_2	β_3
Predominant $G\alpha$ isoform coupled	$G\alpha_q$	$G\alpha_i$ family ($\alpha_{i1}/\alpha_{i2}/\alpha_{i3}$)	$G\alpha_s$	$G\alpha_s$	$G\alpha_s$	$G\alpha_s$
Effector	$G\alpha_{11}$ family ($\alpha_{11}/\alpha_{14}/\alpha_{15}/\alpha_{16}$)	$G\alpha_o$ family (α_{o1}/α_{o2})		$G\alpha_i$ family ($\alpha_{i1}/\alpha_{i2}/\alpha_{i3}$)	$G\alpha_i$ family ($\alpha_{i1}/\alpha_{i2}/\alpha_{i3}$)	$G\alpha_i$ family ($\alpha_{i1}/\alpha_{i2}/\alpha_{i3}$)
	Activation of PLC	Inhibition of AC	Activation of AC	($G\alpha_s$ mediated)	($G\alpha_s$ mediated)	($G\alpha_s$ mediated)
	Activation of PLD	\downarrow cAMP	\uparrow cAMP	Activation of AC	Activation of AC	Activation of AC
	Activation PLA_2	Inhibition of PKA	Activation of PKA	\uparrow cAMP	\uparrow cAMP	\uparrow cAMP
	Activation of Ca^{2+} channels		Activation of L-type Ca^{2+} channels	Activation of PKA	Activation of PKA	Activation of PKA
	Activation of Na^+/H^+ exchangers			Activation of L-type Ca^{2+} channels	Activation of L-type Ca^{2+} channels	Activation of L-type Ca^{2+} channels
	Modulation of K^+ channels			($G\alpha_i$ mediated)	($G\alpha_i$ mediated)	($G\alpha_i$ mediated)
Common secondary interacting proteins	\uparrow MAPK signaling			Inhibition of AC \downarrow cAMP	Inhibition of AC \downarrow cAMP	Inhibition of AC \downarrow cAMP
	Transglutaminase II gC1qR	Spinophilin β -Arrestins eIF2B α	β -Arrestins AKAPs c-Src	β -Arrestins AKAPs c-Src	Inhibition of PKA	Inhibition of PKA ?
Major dimerization partners (365)	α_{1A}/α_{1B} $\alpha_{1B}/\text{Histamine } H_1$ α_{1B}/α_{1D} $\alpha_{1D}/\beta_2\text{-Adrenoceptor}$	$\alpha_{2A}/\beta_1\text{-adrenoceptor}$ $\alpha_{2A}/\mu\text{-OPR}$	$\beta_1/\beta_2\text{-adrenoceptor}$ $\beta_1/\alpha_{2A}\text{-adrenoceptor}$	$\beta_2/\delta\text{-OPR}$ $\beta_2/\kappa\text{-OPR}$ $\beta_2/\beta_3\text{-adrenoceptor}$ $\beta_2/M71\text{-OPR}$ $\beta_2/\alpha_{1D}\text{-adrenoceptor}$ $\beta_2/\beta_1\text{-adrenoceptor}$	$\beta_3/\beta_2\text{-adrenoceptor}$	$\beta_3/\beta_2\text{-adrenoceptor}$
Primary locations	Heart Vessels Smooth muscle	Coronary CNS Vessels Pancreas Platelets	Heart Skeletal muscle	Heart Lung Vessels Kidney Skeletal muscle	Heart Lung Vessels Kidney Skeletal muscle	Adipose tissue Heart
Primary effect on skeletal muscle	Vasoconstriction of large resistance arterioles (134, 336)	Vasoconstriction of small precapillary arterioles (134, 336)	?	Hypertrophy	Hypertrophy	?
Common agonists	Cirazoline M-6434	Clonidine UK14304	Xamoterol Prenalterol	Clenbuterol Fenoterol	Clenbuterol Fenoterol	BRL 37344 CGP12177
	Methoxamine Phenylephrine	BHT-920	Denopamine	Formoterol Salbutamol Salmeterol	Formoterol Salbutamol Salmeterol	
Common antagonists	Corynanthine Prazosin Phentolamine	Yohimbine Rauwolscine	CGP20712A Alprenolol	ICI-118,551 Butoxamine	ICI-118,551 Butoxamine	SR59230A

AKAP, A kinase anchoring protein; MAPK, mitogen-activated protein kinase; PL, phospholipase; AC, adenylyl cyclase; OPR, opioid peptide receptor; PKA, protein kinase A. For further details the reader is referred to Refs. 44, 60, 151, 255, 316, 352, 379, 409, 425, 429, 465.

90, 129, 161, 187, 228, 268, 270, 271, 274, 278, 279, 281, 298, 386, 387, 389, 480, 484). However, very little work has focused on the role of this pathway in normal muscle growth and development, muscle fiber regeneration after injury, or its involvement in pathological conditions where muscle wasting and weakness are indicated. Thus the aim of this review will be to describe the potential role(s) of this novel signaling pathway in skeletal muscle structure and function, with particular emphasis on the β_2 -adrenoceptor, which is the predominant skeletal muscle subtype (226, 370).

A. The Guanine Nucleotide-Binding Regulatory Proteins

All adrenoceptors belong to the guanine nucleotide-binding G protein-coupled receptor (GPCR) family, the

largest group of cell-surface receptors in mammals and which comprise >1% of the human genome (142). The most well-characterized family of GPCRs is the rhodopsin receptors, which include the dopaminergic, adenosine, histamine, α -, and β -adrenergic receptors (142, 426). One of the defining features of the GPCR superfamily is that all of the receptors couple to heterotrimeric guanine nucleotide-binding regulatory proteins (G proteins). These molecules received their name from the typical three subunit composition (designated " $\alpha\beta\gamma$ ").

All GPCRs (including adrenoceptors) have a similar structure, with a conserved structure of seven transmembrane α -helices forming three extracellular loops, including an NH_2 terminus, and three intracellular loops, including a $COOH$ terminus (207, 316). The third-fifth intramembranous regions are believed to be important in ligand

binding, while the third intracellular loop of the GPCR has a central role in G protein coupling (Fig. 1) (207).

The G proteins are located in the cytoplasmic space and act intracellularly, interacting with an intracellular loop of the GPCR. The G protein $\beta\gamma$ subunits ($G\beta\gamma$) form a tightly interacting dimer that is bound to the intracellular plasma membrane via an isoprenyl moiety located on the COOH terminus of the γ -subunit, whereas the G protein α -subunit ($G\alpha$), in its inactive state, remains attached to the $G\beta\gamma$ dimer (48). Activation of the GPCR causes a profound change in the conformation of the intracellular loops and uncovers a previously masked G protein binding site (136, 229, 303). Specifically, the third intracellular loop of the GPCR is involved in G protein binding (233). Upon binding of a ligand to the GPCR, GDP is released from the $G\alpha$ subunit, and subsequent GTP binding occurs, which activates the $G\alpha$ subunit and also exposing effector-interaction sites in the $G\beta\gamma$ dimer (48, 150, 172, 380).

Given that there are at least 27 $G\alpha$ -, 7 $G\beta$ -, and 13 $G\gamma$ -subtypes, there are a large number of $G\alpha\beta\gamma$ combinations that control GPCR signaling (262, 449). In addition, both the GTP-bound $G\alpha$ and the $G\beta\gamma$ dimer are capable of activating downstream effectors (Fig. 2), thus further increasing the complexity of this signaling pathway (106, 239, 262, 458). Endogenous G protein expression is typically much higher than that of GPCRs or effectors, which suggests that most GPCR-G protein-effector signaling is limited by the expression and/or activity of the GPCR rather than that of the G protein (309).

The $G\alpha$ subunits can be divided into four main families, based on their primary sequence: $G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_{q/11}$, and $G\alpha_{12}$, which regulate the activity of many different

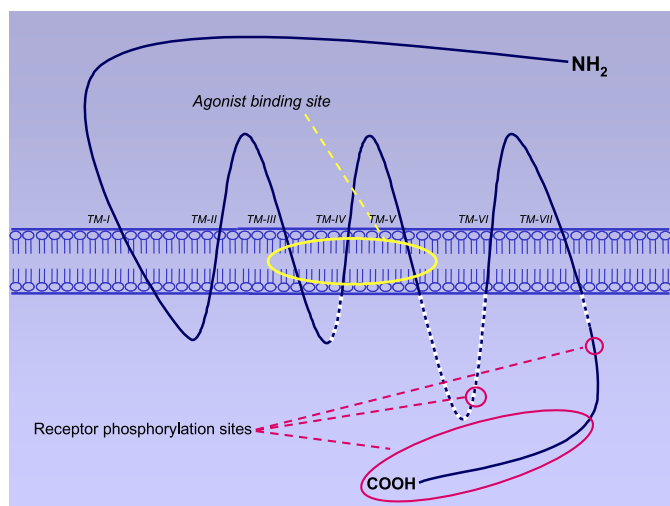
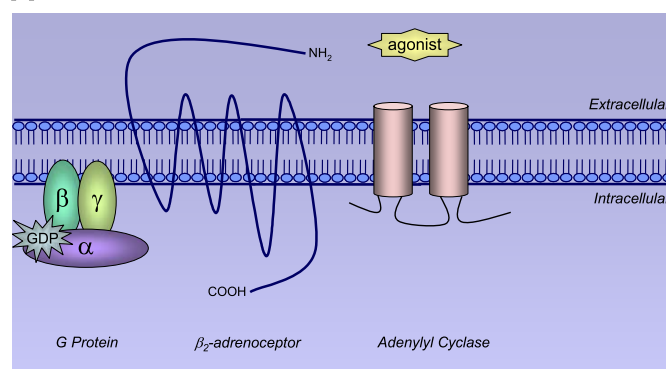


FIG. 1. Basic structure of the seven transmembrane spanning β_2 -adrenoceptor. Regions of agonist binding and receptor phosphorylation are indicated, while the dotted region of the receptor indicates the $G\alpha$ binding domain (207, 257).

A



B

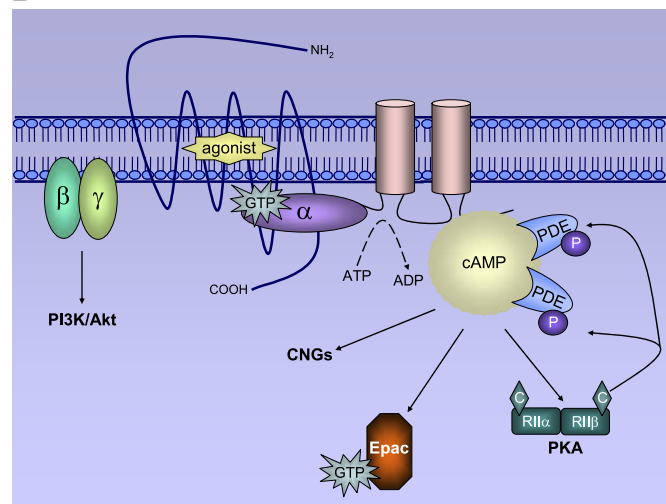


FIG. 2. A: the general β_2 -adrenoceptor signaling pathway involves the receptor, a heterotrimeric G protein, and the membrane-bound adenylyl cyclase. B: activation of the β_2 -adrenoceptor occurs through the binding of an appropriate agonist, which in turn results in the association of a heterotrimeric G protein with the third intracellular loop of the β_2 -adrenoceptor. This association results in GTP displacement of GDP from the α -subunit of the G protein, causing a conformational change in the heterotrimeric protein such that both the α - and $\beta\gamma$ -subunits can activate downstream signaling targets including the PI3K/Akt, protein kinase A (PKA), Epac, and cyclic nucleotide-gated (CNG) signaling pathways (103, 108, 320, 431). The cAMP signal is terminated through its hydrolyzation to 5'-AMP by phosphodiesterases (PDEs) (339).

second messenger systems (262, 460). β -Adrenoceptors have been found to couple predominantly with $G\alpha_s$ and $G\alpha_i$ isoforms to initiate downstream effector pathways including adenylyl cyclase (AC), transmembrane protein kinases, and phospholipases (106, 458).

The complexity of the G protein-GPCR signal-transduction system is only beginning to be appreciated (86, 138, 150, 157, 263, 319, 375, 425, 468, 486), and recent work has demonstrated that GPCRs do not simply function to generate second messenger signals, but rather activate a wide range of signaling proteins and pathways (106, 311, 422).

B. The α -Adrenoceptors

This review focuses primarily on β -adrenoceptor signaling, since the β -adrenoceptors are the predominant subtype in skeletal muscle (226, 370). However, a sparse population of α -adrenoceptors has been identified in skeletal muscle and is usually expressed in higher proportions in muscles that are highly vascularized (370). Therefore, α -adrenergic signaling will only be discussed briefly. For a more detailed discussion on α -adrenergic signaling, the reader is directed to several excellent reviews (394, 409, 429).

The α -adrenoceptors can be activated by catecholamines either via neural synapses or the circulation and are responsible for mediating a diverse array of physiological effects (250). Two major families of α -adrenoceptors have been identified: α_1 - and α_2 -, which are further subdivided into six subtypes α_{1A} -, α_{1B} -, α_{1D} -, α_{2A} -, α_{2B} -, and α_{2C} -adrenoceptors (Table 1).

The α_1 -adrenoceptors couple predominantly to the $G_{q/11}$ family of G proteins and play an important role in the vasoconstriction of large resistance arterioles, blood pressure regulation, and cardiac adaptation to stress (409). Activation of this family of receptors initiates numerous secondary signaling pathways, including members from the phospholipase family, C (PLC), D (PLD), and A_2 (PLA_2), as well as activating Ca^{2+} channels, Na^+/H^+ exchangers, and K^+ channels (Table 1) (350, 404).

Signaling via the α_2 -adrenoceptors is mediated predominantly via the $G_{i/o}$ family of G proteins, although signaling via G_{α_s} and G_{α_q} has been observed after stimulation of α_{2A} -adrenoceptors (79). The α_2 -adrenoceptor family is a critical regulator of a diverse range of physiological events, including neurotransmitter release, cardiovascular performance, and the response to sedation, anesthetics, or analgesics (1, 182, 250, 260, 429). These responses are regulated through a variety of downstream signaling pathways, including the inhibition of AC, suppression of L-type Ca^{2+} channels, and activation of the mitogen-activated protein kinase (MAPK) pathway (429, 451).

As skeletal muscle blood flow is under autonomic neural control, predominantly through sympathetic innervation (249, 282), it is not surprising that sympathetic vasoconstriction is regulated via both α_1 - and α_2 -adrenoceptors, with each receptor believed to play a distinct role in the control of muscle blood flow. Stimulation of the α_1 -adrenoceptors elicits vasoconstriction of large resistance arterioles, while activation of the α_2 -adrenoceptors causes constriction of the small precapillary arterioles (134, 336).

C. The β -Adrenoceptors

β -Adrenoceptors play a regulatory role in cardiovascular, respiratory, metabolic, and reproductive function. Therefore, it is not surprising that the β -adrenoceptor family is the most widely studied of all the adrenergic receptors. Three subtypes of β -adrenoceptors have been identified and cloned: β_1 -, β_2 -, and β_3 -adrenoceptors (116, 130, 145, 465), each with a 65–70% homology in their amino acid composition (232). The β -adrenoceptor family was originally believed to signal predominantly via coupling with G_{α_s} ; however, more recent studies suggest that both β_2 - and β_3 -adrenoceptors are also capable of coupling to G_{α_i} (157, 467, 468). The crystal structure of the β_2 -adrenoceptor has been described in two studies (85a, 369a).

The most well-documented β -adrenoceptor signaling pathway involves the cAMP-protein kinase A (PKA) signaling pathway (Fig. 2), which has been characterized in numerous cell types and tissue systems (431). In skeletal muscle, activation of this pathway is believed to be, at least in part, responsible for the anabolic response of skeletal muscle to β -adrenoceptor stimulation.

III. β -ADRENOCEPTOR SIGNALING IN SKELETAL MUSCLE

Much of our current knowledge of β -adrenoceptor signaling in skeletal muscle is based on work conducted in cardiac muscle (379, 399, 467–469). It is only recently that we have begun to appreciate the importance of this system in skeletal muscle growth, development, and repair after injury (31, 187, 272).

A. Skeletal Muscle β -Adrenoceptor Subtypes

Skeletal muscle contains a significant proportion of β -adrenoceptors, which are mostly of the β_2 -subtype, but there are ~7–10% β_1 -adrenoceptors present (226, 462) as well as a smaller population of α -adrenoceptors, usually found in higher proportions in slow-twitch muscles (370). Slow-twitch muscles, such as the soleus muscle, have a greater density of β -adrenoceptors than fast-twitch muscles, such as the extensor digitorum longus (EDL) (283, 386, 387). Although the functional significance of this difference in β -adrenoceptor density is not yet fully understood, the response to β -agonist administration appears to be greater in fast- than in slow-twitch skeletal muscles (386, 389).

Recently, a fourth β -adrenoceptor subtype (designated as a “putative” β_4 -adrenoceptor) was proposed to exist in the mouse (216, 217) but was later reclassified as a novel β_3 -adrenoceptor isoform (designated as β_{3a} - and β_{3b} -adrenoceptors) (133). Both β_3 -adrenoceptors are be-

lieved to have stimulatory effects that are mediated through a $G\alpha_s$ pathway (215, 216, 392). The β_{3b} -adrenoceptor is also believed to be coupled to a second pathway involving the inhibitory G protein, $G\alpha_i$ (195).

One of the most important characteristics of adrenoceptors is that a catecholamine can elicit very different responses depending on the adrenoceptor that it activates (Table 1). This enables the adrenoceptors to be targeted specifically and to be manipulated by synthetically tailored agents. Many synthetic adrenoceptor agonists and antagonists have been developed for the treatment of (primarily) cardiovascular diseases. β -Adrenoceptors have been the main focus of most investigations regarding adrenergic receptors (333), and information gathered from this receptor has helped develop a family of β_2 -adrenoceptor agonists (β_2 -agonists) for clinical purposes (25, 453).

Since the β_2 -adrenoceptor is the predominant subtype in skeletal muscle, the remainder of this review will focus on the β_2 -adrenoceptor signaling pathways in skeletal muscle. Signaling via the β_1 - and β_3 -adrenoceptors will be discussed where appropriate.

B. G Protein Coupling in Skeletal Muscle

While β_2 -adrenoceptor-mediated signaling has been traditionally believed to involve selective coupling to $G\alpha_s$ to initiate downstream signaling via AC pathways (Fig. 2), recent studies suggest that the β_2 -adrenoceptor may exhibit dual coupling to both $G\alpha_s$ and $G\alpha_i$ (466). This dual coupling mechanism has been described in numerous studies involving cardiac muscle (222, 467), and more recently in skeletal muscle (157). In addition to the well-documented inhibition of AC activity (3, 465, 466), β_2 -adrenoceptor coupling to $G\alpha_i$ appears to activate $G\alpha_s$ independent pathways (98, 486).

Much of our current understanding of β_2 -adrenoceptor-to- $G\alpha_i$ coupling is based on the work of Dr. Rui-Ping Xiao, who provided the first direct biochemical evidence for the interaction of β_2 -adrenoceptor and $G\alpha_i$ in cardiac muscle (467). In subsequent studies, $G\alpha_i$ was found to be essential for the spatial localization and effector selectivity of the $G\alpha_s$ -stimulated cAMP response (82, 243).

To further complicate skeletal muscle β_2 -adrenoceptor signaling, the $G\beta\gamma$ dimer has been found to initiate intracellular signaling pathways independent of the $G\alpha$ subunit (471). Specifically, $G\beta\gamma$ activates the phosphoinositol 3-kinase (PI3K)-AKT signaling pathway (Fig. 2) (263, 471). PI3K is thought to phosphorylate the membrane phospholipid phosphatidylinositol-4,5-bisphosphate (PIP_2), generating phosphatidylinositol-3,4,5-trisphosphate (PIP_3), and creating two lipid-binding sites on the cell membrane for the serine/threonine kinase AKT (also referred to as protein kinase B) and 3'-phosphoinositide-dependent pro-

tein kinase 1 (PDK). AKT is phosphorylated at the membrane by PDK, and once activated, AKT phosphorylates numerous proteins involved in protein synthesis, gene transcription, cell proliferation, and survival (50, 230, 343, 381). These pathways are described in detail in section IIIH.

C. cAMP

The $G\alpha_s$ -AC-cAMP is the most well characterized of the β_2 -adrenoceptor signaling pathways and is generally thought to be, at least partially, responsible for the β_2 -adrenoceptor-mediated hypertrophy in skeletal muscle (187, 328). However, since cAMP is involved in a myriad of cellular processes, including the regulation of the cell cycle, proliferation and differentiation, regulation of intracellular transport mechanisms, and chromatin condensation and decondensation, there are numerous regulatory mechanisms in place such that the second messenger actions of cAMP are regulated tightly both spatially and temporally (431).

1. Adenylyl cyclase

Adenylyl cyclases catalyze the conversion of ATP to cAMP and therefore play an important role in β_2 -adrenoceptor-mediated signaling. There are at least nine distinct membrane-bound isoforms of AC (AC1-AC9), in addition to one soluble form (sAC) (173). All AC isoforms exhibit a similar structure of a variable NH_2 -terminal region, two hydrophobic transmembrane domains, and two cytoplasmic (catalytic) domains (Fig. 2).

Each of the membrane-bound AC isoforms exhibits a basal level of activity, which is generally increased after binding with a stimulatory $G\alpha$ protein, or decreased after binding with an inhibitory $G\alpha$ protein (173). However, the response of specific AC isoforms to other regulatory proteins can vary dramatically. For example, protein kinase C (PKC) inhibits the activity of AC6, whilst increasing the activity of the remaining AC isoforms (173, 203). In addition, the $G\beta\gamma$ dimer inhibits the activity of AC1, AC5, and AC6, whilst increasing the activity of AC2, AC4, and AC7 (173, 430).

Skeletal muscle has been found to contain predominantly AC2 and AC9, with measurable levels of the ubiquitously expressed AC6 and AC7 (173, 428, 441). Interestingly, skeletal muscle does not express a detectable amount of AC5, the predominant isoform in cardiac muscle (131), indicating that subtle differences in β_2 -adrenergic mediated signaling exist between the two tissues.

The potential role of AC in mammalian skeletal muscle growth and development was first identified by Suzuki et al. (428), who demonstrated that compared with adult mouse muscle, skeletal muscle from the neonate expressed higher levels of the ubiquitously expressed AC6

and AC7, and lower levels of AC2 and AC9. Although the consequences of different isoform expression remain to be elucidated, these findings implicate AC in normal skeletal muscle growth and development.

2. Phosphodiesterases

The cyclic nucleotide phosphodiesterase (PDE) superfamily comprises 21 genes and their multiple splice variants give rise to more than 50 different PDE proteins (449). All mammalian PDEs comprise a similar modular architecture: an allosteric regulatory NH₂-terminal domain, a highly conserved central catalytic domain, and targeting domains that have only been identified recently (132, 141). PDEs are the enzymes responsible for the hydrolysis (degradation) of cyclic nucleotides (including cAMP) into 5'-AMP (339, 431). Thus the intracellular concentration of cAMP is determined via the balance between cAMP production by AC, and cAMP degradation by PDEs (Fig. 2). Skeletal muscle contains numerous isoforms of PDE, including PDE4, PDE7, and PDE8 (46, 184, 339). However, PDE4 appears to contribute to the majority of cAMP hydrolyzation (46).

PDEs are phosphorylated by PKA, which leads to their activation and subsequent hydrolysis of cAMP (118). Thus PKA has a negative-feedback mechanism that limits the temporal expression of cAMP. In addition to PKA, PDE activity is regulated by members of the MAPK family, specifically extracellular signal-regulated kinase (ERK), which has been found to decrease the activity of PDEs in cell culture experiments (119, 190).

Selective PDE inhibitors have been developed and trialed clinically for a diverse range of pathological conditions (284, 371). A nonselective PDE inhibitor (pentoxifylline), or one of two different selective PDE4 inhibitors (cilomilast and rolipram), has been administered to mice undergoing muscle atrophy, with the aim of reducing skeletal muscle cAMP degradation, and thus increasing cAMP-mediated muscle hypertrophy (186). The selective PDE4 inhibitor rolipram was found to prevent the muscle wasting and weakness associated with sciatic nerve resection and limb casting. Interestingly, rolipram did not alter the mass of control muscles (186).

In another study, Lira et al. (261) administered either pentoxifylline or isobutylmethylxanthine (a member of the methylxanthine family) to septic rats and demonstrated that treatment with either compound increased muscle cAMP levels and reduced skeletal muscle proteolysis. In treated rats, the increase in tumor necrosis factor- α (TNF- α) observed with sepsis was completely inhibited. These findings indicate that cAMP signaling may play an important role in preventing proteolysis, but may not alter the rate of protein synthesis (327).

Several reports have documented a change in the PDE activity of dystrophic skeletal muscle (45, 46, 69).

The muscular dystrophies are generally characterized by progressive muscle wasting and weakness and exhibit a high level of muscle degeneration (189, 455). In dystrophic *mdx* mice, total PDE activity was increased in young (<5 wk old) mice and decreased in older (>15 wk old) mice (45). This suggests a disruption in the balance between cAMP production and degradation in dystrophic skeletal muscle which might have detrimental effects on downstream signaling pathways involved in muscle fiber regeneration.

D. Downstream Effectors of cAMP

The production of cAMP results in the activation of numerous downstream signaling pathways, including the well-described PKA signaling pathways, as well as the novel cAMP targeted exchange protein activated directly by cAMP (Epac, also referred to as cAMP regulated guanine nucleotide exchange factors) and the cyclic nucleotide-gated (CNG) cation channels (Fig. 2) (52, 120, 342, 349, 431). The PKA signaling pathway is the most commonly examined β -adrenoceptor effector in skeletal muscle (90, 326, 327). However, with the discovery of Epac, the possibility of PKA-independent signaling pathways cannot be ignored (56, 368). cAMP activation of CNGs appears to be limited to specialized cells, such as olfactory neurons (103).

1. PKA

PKA is the most commonly studied effector molecule of cAMP. Mammalian PKA is a heterotetramer composed of two regulatory (R) and two catalytic (C) subunits. There are two isoforms of PKA: PKA I and PKA II. While both isoforms are ubiquitously expressed and play an important role in cell metabolism and growth (120), PKA I (containing a RI α and RI β heterodimer and two C subunits) is primarily cytoplasmic and more sensitive to cAMP, whereas PKA II (containing a RII α and RII β heterodimer and two C subunits) is associated with particulate subcellular fractions (294, 307). cAMP is required to bind to two sites on each of the R-subunits before a conformational change results in the dissociation of the R-subunits from the active C-subunit (C α , C β , or C γ) (236, 431). The active C-subunits then phosphorylate various serine and threonine residues on specific substrate proteins which initiate multiple signaling pathways discussed in detail in section III G (Fig. 3A).

2. Exchange protein activated directly by cAMP

The discovery of Epac, a guanine nucleotide exchange factor for the Ras-like small GTPases Rap1 and Rap2, has opened up an entirely novel signaling pathway activated by cAMP (Fig. 3B). Currently, two separate

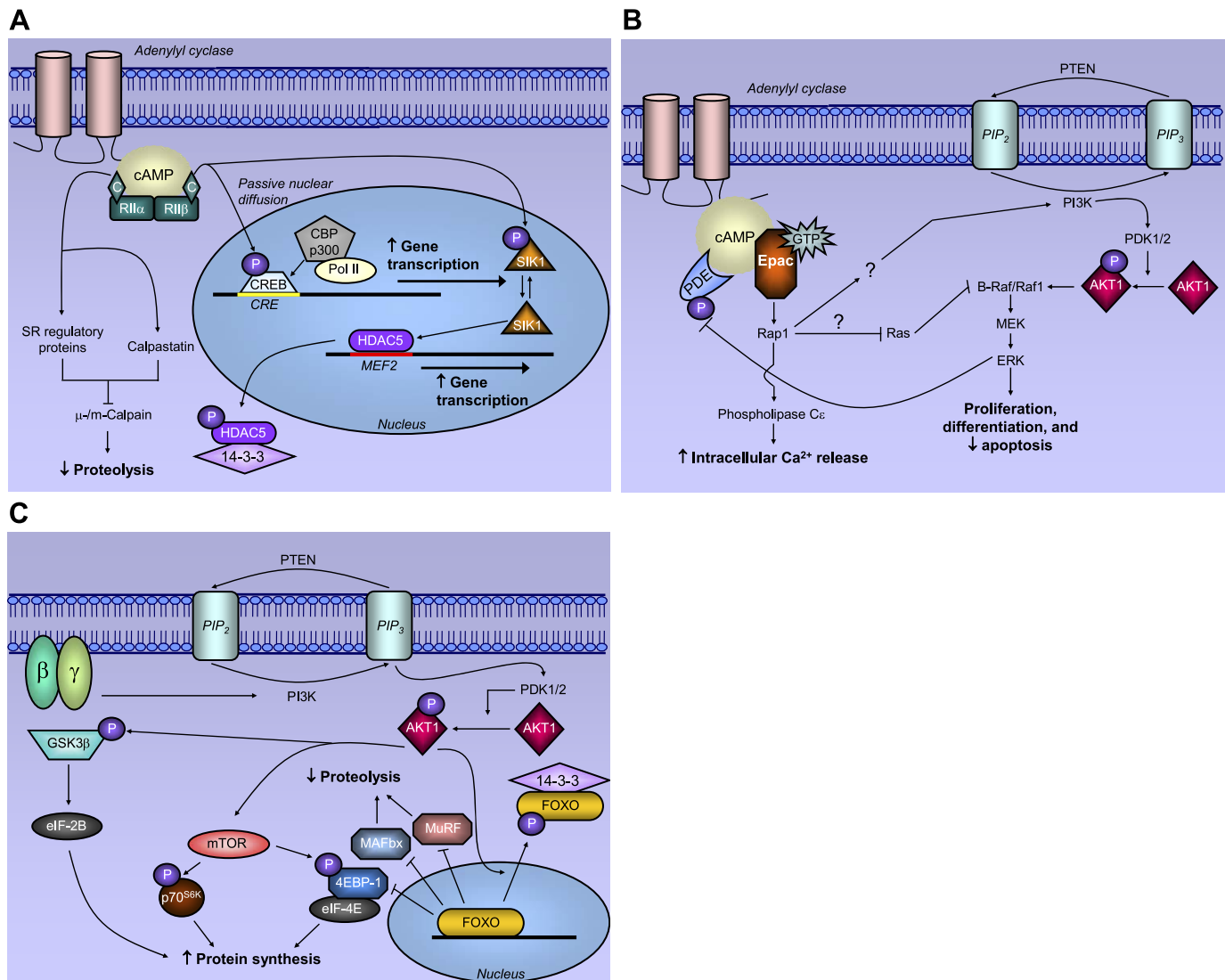


FIG. 3. *A*: PKA-mediated signaling pathways in skeletal muscle. Binding of cAMP to the regulatory subunits (R) of PKA releases the active catalytic subunits (C), which can either directly bind to and phosphorylate target proteins or enter the nucleus via passive diffusion. Active PKA C-subunits can initiate transcription directly via the DNA binding of CREB, or indirectly through the phosphorylation of HDAC and subsequent activation of MEF2 transcription (40, 72, 292). *B*: in addition to the well-documented PKA signaling pathway, cAMP can bind to the novel protein Epac and, through the small GTPase Rap1, alter mitogen-activated protein kinase (MAPK) signaling (107, 355, 356). *C*: the G $\beta\gamma$ dimer is also believed to play an important role in β_2 -adrenoceptor signaling in skeletal muscle (138, 413). This protein dimer is believed to activate PI3K/AKT signaling pathway. Phosphorylation of AKT is known to have numerous downstream effects, including the phosphorylation and subsequent inhibition of GSK3 β , thus activating eIF-2B and increasing protein synthesis; activation of mTOR resulting in increased protein synthesis via phosphorylation and activation of p70^{S6K} pathways, and phosphorylation of 4EBP-1 and subsequent activation of eIF-4E; and the phosphorylation and subsequent nuclear exclusion of FOXO1, FOXO3a, and/or FOXO4 (which binds 14-3-3 and remains in the cytosol) which prevents the transcription of atrophic genes such as MAFbx and MuRF, and genes involved in the inhibition of protein synthesis such as 4EBP-1 (50, 230, 381, 418).

isoforms of Epac have been identified, Epac1 and Epac2. Epac1, first identified by de Rooij et al. (108), is ubiquitously expressed and has a single cAMP binding site, whereas Epac2 contains a second cAMP binding site and is localized to the brain and adrenal glands (107).

Our knowledge regarding the molecular mechanisms and function of Epac stimulated Rap activation is limited, with only a handful of studies (so far) investigating the role of this novel signaling pathway (52). Rap1 has been identified to play an important role in the regulation of

integrins (53), a family of cell-surface molecules that regulate cell adhesion to the extracellular-matrix, and cadherins (364). In contrast, Rap2 has been implicated in Ca²⁺ release in β -cells of the pancreas (212).

β -Adrenoceptor signaling has been found to activate the Epac signaling pathway in skeletal muscle (56). β -Adrenoceptor-induced activation of Epac potentiated the cellular response to insulin, likely via Rap1-mediated signaling to PI3K and AKT (Fig. 3*B*), highlighting the possibility that physiological responses to β -adrenoceptor

stimulation previously attributed to PKA may occur via the Epac signaling pathway (56).

Shi et al. (403) demonstrated that β -adrenoceptor stimulation activated the ERK signaling pathway in skeletal muscle (403). As β -adrenoceptor stimulation leads to Epac-mediated Rap1 activation in skeletal muscle (56), and Rap1 initiates ERK signaling in myoblasts (355, 356), it is possible that the cAMP/Epac/Rap1/ERK signaling pathway plays a previously unidentified role in the skeletal muscle response to β -adrenoceptor stimulation (Fig. 3B).

E. Localization of cAMP Signaling: A Kinase Anchoring Proteins

Compartmentalization and localization of cAMP-mediated signals was first hypothesized by Stanley Keely (218, 219), who demonstrated that both norepinephrine and prostaglandin E_1 increased the concentration of cAMP in cardiac myocytes, but only norepinephrine stimulated contraction. Since these seminal studies, live-cell imaging and whole cell patch-clamping experiments have confirmed the existence of localized pools of cAMP within numerous cell types (210, 477).

The localization of cAMP signaling to specific substrates is achieved through the actions of scaffolding proteins, such as A kinase anchoring proteins (AKAPs), which bind numerous cAMP effector molecules including PKA, Epac1, PDEs, ERK, and protein phosphatases, such as calcineurin (118, 294). There are currently more than 50 AKAPs that have been described, and all share the ability to bind PKA. PKA is anchored to AKAP via an interaction between the NH_2 termini of the R-subunit dimer of PKA and a 14- to 18-amino acid amphipathic α -helix region of AKAP (28, 112). In addition, each AKAP contains a unique subcellular targeting region containing spectrin repeat domains that localize its expression to specific regions within the cell (Fig. 4) (307). AKAP predominantly binds the RII subunits of PKA, although several AKAPs bind the RI subunits with high affinity (9). Thus, following cAMP activation, PKA linked to AKAP will initiate a response localized to the AKAP-targeted region.

In addition to binding PKA, it is becoming clear that many AKAPs also bind PDEs, thus creating a local negative-feedback system for the initiating cAMP signal. Thus, in addition to regulating PKA signaling in a spatial manner, AKAPs act to limit PKA signaling temporally (28). Furthermore, AKAPs form multiprotein complexes, which integrate multiple downstream signaling pathways.

Skeletal muscle contains multiple isoforms of AKAP, including muscle AKAP (mAKAP, formerly AKAP100), AKAP350 (also known as yotiao, AKAP450 and AKAP9), AKAP95, and AKAP149 (294). mAKAP is the most com-

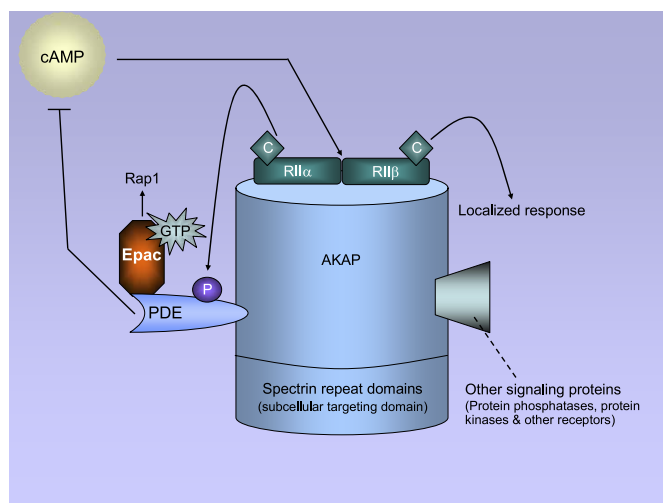


FIG. 4. The localization of cAMP signaling to specific substrates is mediated via the actions of scaffolding proteins such as AKAPs. The regulatory subunits of PKA are bound to a 14- to 18-amino acid amphipathic α -helix region of AKAP, which is itself bound, via spectrin repeat domains, to a subcellular targeting region. In addition, AKAPs can bind PDEs, creating temporal control over the cAMP-induced PKA signal. Finally, AKAPs can bind other signaling proteins, including protein phosphatases (such as calcineurin), protein kinases, and other receptors (such as the RyR) (28, 118, 294).

monly studied AKAP in skeletal and cardiac muscle and has been identified at the perinuclear membrane and the sarcoplasmic reticulum (SR, a large internal storage site of Ca^{2+}) (293). The observation that mAKAP is localized to the SR indicates that it may play a role in the targeted phosphorylation of proteins and other receptors in and around the SR. Potential targets include the ryanodine receptor (RyR), protein phosphatases, and protein kinases (Fig. 4) (294, 307, 385).

No study to date has examined the role of mAKAP in skeletal muscle wasting disorders. Due to the importance of this protein in localizing the PKA signal to the SR, it would be interesting to hypothesize that disruption of this protein could significantly impair the Ca^{2+} release response to β -adrenoceptor stimulation.

F. PKA Regulation of Intracellular Calcium Signaling

Muscle fiber activation and force production, a process termed excitation-contraction (EC) coupling (247, 248), involves the shortening of sarcomeres in response to stimulation via an action potential (AP). Skeletal muscle contraction is initiated when an AP is transmitted from the brain to a motoneuron, via the spinal cord, and to the muscle fiber via the neuromuscular junction (NMJ). When an AP reaches the NMJ, the electrical stimulus elicits exocytotic release of the neurotransmitter ACh into the synaptic cleft (65, 135). ACh molecules bind to ligand-

gated channels located on the muscle fiber membrane beneath the NMJ, enabling Na^+ to flow down a concentration gradient and propagate along the surface membrane of the muscle fiber and its tubular invaginations, known as transverse tubules (t tubules). Voltage-sensitive Ca^{2+} channels adjacent to the t tubules, termed dihydropyridine receptors (DHPRs), stimulate Ca^{2+} release from the SR into the cytoplasm via specialized Ca^{2+} release channels (RyRs), which diffuses throughout the filamentous structure.

As described previously, PKA is bound to the RyR via mAKAP. Reiken et al. (374) have demonstrated that activation of PKA leads to phosphorylation of skeletal muscle RyRs at Ser²⁸⁴³ resulting in a dissociation of FKBP12 from the RyR and therefore increasing the open probability of the channel (58, 287, 374). These results indicate that in addition to potentiating Ca^{2+} -mediated contraction (discussed further in sect. III), β -adrenoceptor activation may activate signaling pathways mediated via the formation of Ca^{2+} microdomains, including Ca^{2+} /calmodulin-dependent kinase (CaMK), PKC, and calcineurin (73, 337, 378).

As well as targeting SR regulatory proteins and altering Ca^{2+} -mediated signaling, the C-subunit of PKA can phosphorylate calpastatin which leads to the inhibition of the proteolytic actions of the enzymes "calpains" (178, 327, 374). Two ubiquitously expressed calpains exist in skeletal muscle, μ -calpain (low Ca^{2+} -activated) and m-calpain (high Ca^{2+} -activated). Inhibition of either of these enzymes by phosphorylated calpastatin can reduce the level of Ca^{2+} -dependent proteolysis (327, 436, 437). Calpains are concentrated in the Z-disc and are involved in myofibril protein degradation, specifically in the disassembly of the myofibril (240). In conditions where muscle wasting predominates (e.g., muscular dystrophies, sepsis, and cancer cachexia), calpains have been implicated in the rate of protein turnover (101, 436).

G. PKA-Mediated Skeletal Muscle Growth and Development

Following cAMP activation of PKA, the C-subunits of PKA are thought to phosphorylate and regulate the activity of numerous proteins (Fig. 3A). In addition, free C-subunits of PKA are capable of diffusing passively into the nucleus, where they can regulate the expression of many target genes via direct phosphorylation of the cAMP response element (CRE) binding protein (CREB), or via a modulator that acts on second-generation target genes (72, 292).

The CRE binding protein is a nuclear transcription factor that is ubiquitously expressed and has been implicated in many processes, including cell proliferation, dif-

ferentiation, adaptation, and survival (292). CREB forms a homodimer and binds to a conserved CRE region on DNA. Activation of PKA by cAMP and subsequent nuclear entry of the C-subunit of PKA phosphorylates CREB at a single serine residue site (Ser¹³³) (170). Phosphorylation of Ser¹³³ promotes transcription at the CRE region through recruitment of the transcriptional coactivators CREB-binding protein (CBP) and p300, which mediate transcriptional activity through their association with RNA polymerase II (Fig. 3A) (155, 292). CREB-phosphorylation promotes activation of genes containing a CRE-region, of which there are >4,000 in the human genome (360, 485). Finally, CRE-gene activation is terminated by dephosphorylation of CREB, a process regulated by the serine/threonine phosphatases PP1 and PP2A (169, 452).

One recently identified target for β -adrenoceptor-mediated CRE activation in skeletal muscle is the promoter region of the orphan nuclear receptor, NOR-1 (NR4A3) (335, 346). β_2 -Adrenoceptor activation is associated with an increased expression of NOR-1 and the related orphan nuclear receptor nur-77 (NR4A1) (291, 346). Interestingly, Pearen et al. (346) have found that siRNA-mediated inhibition of NOR-1 expression was associated with a dramatic increase (>65-fold) in the levels of myostatin mRNA in C2C12 cells. Myostatin is a member of the transforming growth factor- β superfamily and a potent negative regulator of muscle mass (302). These results indicate that β -adrenoceptor activation, through increased NOR-1 expression, may inhibit myostatin expression and thus promote skeletal muscle growth.

The transcriptional adapters CBP and p300 promote skeletal muscle myogenesis via the coactivation of a number of myogenic basic helix-loop-helix (bHLH) proteins (127, 301, 393). The family of myogenic bHLH proteins, including MyoD, myogenin, myf5, and MRF4, activate muscle gene transcription via pairing with the ubiquitously expressed E-box consensus sequence in the control regions of muscle-specific genes (301, 313). Sartorelli et al. (393) demonstrated that p300 and CBP may positively influence myogenesis by acting as a "bridge" between the myogenic bHLH and the myocyte enhancer factor 2 (MEF2) family of proteins.

In addition to transcriptional coactivation, CBP and p300 have intrinsic histone acetyltransferase (HAT) activity (155, 382, 435). HATs are believed to play an important role in transcription, as they catalyze the transfer of acetyl groups from acetyl-coenzyme A to the ϵ -amino group of lysine side chains of specific proteins, including several transcriptional regulatory proteins (473). Therefore, the actions of CBP and p300 could increase the accessibility of docking sites for transcriptional proteins and regulators (334, 435).

Chen et al. (83) identified an unexpected role for PKA/CREB signaling during myogenesis. It was proposed

that myogenic gene expression of Pax3, MyoD, and Myf5 is dependent on AC/cAMP-mediated phosphorylation of PKA and subsequent activation of CREB. The authors demonstrated the importance of CREB in the developing myotome, since CREB^{-/-} mice did not express Pax3, MyoD, or Myf5 and myotome formation was defective (83). It remains to be seen whether β -adrenoceptor-mediated activation of PKA/CREB signaling has a similar response during myogenesis.

Berdeaux et al. (40) demonstrated a novel role of CREB in mediating the activity of the transcription factor MEF2, a family of transcription factors that play a key role in the differentiation of muscle cells (Fig. 3A). In this study, β -adrenergic-stimulated CREB modulated the phosphorylation status of the class II histone deacetylase HDAC5 in mouse skeletal muscle, by increasing the expression of salt-inducible kinase 1 (SIK1). Activated SIK1 phosphorylated HDAC5 resulted in its nuclear exclusion and subsequent activation of the MEF2 myogenic program (40). These exciting results demonstrate the complexity of the downstream activators of the β -adrenergic signaling pathway and highlight the previously unappreciated role of this pathway in skeletal muscle.

H. PKA Independent Signaling Pathways in Skeletal Muscle

In addition to the well-described G_{α_s} -cAMP signaling pathways, studies have implicated the $G_{\beta\gamma}$ subunits in various cell signaling processes, which may have important roles in β -adrenoceptor signaling in skeletal muscle (Fig. 3C) (104, 106, 115, 138, 311). Specifically, in vitro cell culture experiments have revealed that the G_{α_i} -linked $G_{\beta\gamma}$ subunits activate the PI3K-AKT signaling pathway (263, 319, 320, 397).

The PI3K-AKT signaling pathway has been implicated in protein synthesis, gene transcription, cell proliferation, and cell survival (50, 152, 230, 343, 381). Although there are three distinct isoforms of AKT, the predominant skeletal muscle isoform is AKT1 (324). Activation of PI3K phosphorylates the membrane-bound PIP₂, creating a lipid-binding site on the cell membrane for both AKT1 and PDK. PDK then phosphorylates AKT1 at the membrane (Fig. 3C) (331).

Multiple skeletal muscle AKT1 pathways are activated following β -adrenoceptor stimulation, and these lead predominantly to skeletal muscle hypertrophy (230, 413). Kline et al. (230) found that stimulation of the β -adrenoceptor signaling pathway resulted in AKT phosphorylation and subsequent activation of the mammalian target of rapamycin (mTOR). Initiation of mTOR signaling leads to the phosphorylation and activation of p70^{S6} kinase (p70^{S6K}) and the inactivation of 4EBP-1 (also termed PHAS-1). p70^{S6K} is known to mediate the phosphorylation

of the 40S ribosomal S6 protein, resulting in the upregulation of mRNA translation encoding for ribosomal proteins and elongation factors (205). Inactivation of 4EBP-1 removes its inhibitory action on the protein initiation factor eukaryotic initiation factor 4E (eIF-4E) (Fig. 3C) (246, 325). These results support the previous findings of Sneddon et al. (413) who found an increased phosphorylation of 4E-BP1 and p70^{S6K} in rat plantaris muscle after 3 days of clenbuterol treatment.

Other signaling pathways activated through PI3K-AKT1 phosphorylation include glycogen synthase kinase 3 β (GSK3 β) (50) and the forkhead box O transcription factors FOXO1 (also referred to as FKHR), FOXO3a (FKHRL1), and FOXO4 (AFX) (146, 391, 442). GSK3 β is inactivated by AKT1, resulting in the expression of a dominant negative form of GSK3 β . Since GSK3 β normally acts to inhibit the translation initiation factor eIF2B, blockade of GSK3 β by AKT1 might promote protein synthesis (50, 381).

AKT1 signaling is not only involved in the signaling pathways responsible for muscle hypertrophy, but it has been implicated in the inhibition of signaling pathways responsible for "muscle atrophy." AKT1 inactivation of FOXO leads to nuclear exclusion and inhibition of the forkhead transcriptional program. The DNA displacement and subsequent nuclear exclusion of FOXO requires the involvement of 14-3-3 proteins, which bind to FOXO following AKT1-mediated phosphorylation (442). 14-3-3 proteins are among a family of chaperone proteins that interact with specific phosphorylated protein ligands (442).

Activation of the forkhead transcriptional program is necessary for the induction of both muscle RING finger 1 (muRF1) and muscle atrophy F-box (MAFbx, also called atrogin-1) (391, 424). Both muRF1 and MAFbx encode ubiquitin ligases that function to conjugate ubiquitin to protein substrates and are upregulated in numerous models of muscle atrophy (49, 438). Thus, by phosphorylating and inactivating FOXO, AKT1 blocks the induction of FOXO-mediated atrophy signaling via muRF1 and MAFbx. β -Adrenoceptor activation has been found to reduce the expression of muRF1 and MAFbx in skeletal muscle from denervated and hind-limb-suspended rats, an effect possibly mediated via AKT1-mediated inhibition of the forkhead transcriptional program (230).

It is interesting to note that while FOXO1 has been found to regulate the expression of both MAFbx and muRF1 (424), FOXO3a appears only to activate the MAFbx promoter (391). In addition, while measurable levels of FOXO4 have been identified in skeletal muscle (146), very little is known about its role in skeletal muscle atrophy. Furuyama et al. (146) characterized the expression pattern of FOXO1, FOXO3a, and FOXO4 with aging and caloric restriction in rats. FOXO4 mRNA ex-

pression was found to increase from 3–12 mo and then decrease from 12–26 mo; a similar pattern was observed for FOXO3a expression (146). Interestingly, FOXO1 mRNA expression remained unchanged. In contrast, caloric restriction resulted in an increase in the expression levels of both FOXO4 and FOXO1, but not FOXO3a (146). These results indicate the complexity of the forkhead transcriptional program in the regulation of skeletal muscle atrophy (211).

Southgate et al. (418) identified a novel role for FOXO1 in binding to the promoter region of 4EBP-1 which resulted in increased mRNA and protein expression. Associated with the increase in 4EBP-1 was a reduction in mTOR activation and p70^{S6K}. These important findings indicate that in addition to the previously reported role in atrophic signaling pathways, FOXO1 plays an active role in inhibiting protein synthesis.

A number of researchers have identified genes that are activated by β -adrenoceptor stimulation, but the mechanism for their activation remains unclear. For example, McDanel et al. (295) examined differential gene expression in skeletal muscle after β -agonist administration to evaluate the role of genes thought responsible for muscle growth. Decreased mRNA abundance following β -adrenoceptor stimulation was confirmed for DD143 identified as ASB15, a bovine gene encoding an ankyrin repeat and a suppressor of cytokine signaling (SOCS) box protein, in both cattle and rats (295, 296, 419). The authors reported that ASB15 was a member of an emerging gene family involved in a variety of cellular processes including cellular proliferation and differentiation (295).

Similarly, Spurlock et al. (421) examined gene expression changes in mouse skeletal muscle 24 h and 10 days after β -adrenoceptor stimulation. They identified genes involved in processes important to skeletal muscle growth, including regulators of transcription and translation, mediators of cell-signaling pathways, and genes involved in polyamine metabolism. They reported changes in mRNA abundance of multiple genes associated with myogenic differentiation relevant to the effect of β -adrenoceptor stimulation on the proliferation, differentiation, and/or recruitment of satellite cells into muscle fibers to promote muscle hypertrophy. Similarly, they showed an upregulation of translational initiators responsible for increasing protein synthesis (421).

I. β -Adrenergic-Mediated Apoptosis

Apoptosis, or programmed cell death, is an evolutionarily conserved process involving the activation of highly specific proteolytic enzymes, termed caspases (183). Activation of caspases leads to DNA fragmentation, nuclear condensation, proteolysis, membrane blebbing and cell fragmentation, the formation of apoptotic bodies, and

eventual removal by macrophages (183). Apoptosis is essential for embryogenesis, development, and the maintenance of cell numbers. However, impaired regulation of apoptosis can lead to tumor proliferation or atrophy in the case of skeletal muscle (124, 288).

β -Adrenoceptor signaling has been implicated in apoptosis in numerous tissues; however, there is much debate as to whether this pathway promotes or inhibits apoptosis (62, 64, 99, 487). In the heart, β_1 -adrenoceptor stimulation initiates pathways leading to cardiomyocyte apoptosis, whereas activation of the β_2 -adrenoceptor pathway has been linked with reduced apoptosis through coupling with $G\alpha_i$ (99, 359).

Geng et al. (149) demonstrated that isoproterenol activation of both β_1 - and β_2 -adrenoceptors in mice with a cardiac specific overexpression of $G\alpha_s$ resulted in a greater level of cardiomyocyte apoptosis than in control mice. Other studies have used the $G\alpha_i$ specific inhibitor pertussis toxin (PTX) to demonstrate the antiapoptotic effect of this signaling pathway (99, 359). These findings support the hypothesis that activation of the β -adrenoceptor/ $G\alpha_s$ pathway activates apoptotic signaling in the heart, whereas activation of the β_2 -adrenoceptor/ $G\alpha_i$ pathway inhibits cardiomyocyte apoptosis.

In the forebrain of rats exposed to a period of ischemia, β_2 -adrenoceptor stimulation increased the expression of the antiapoptotic protein Bcl-2 and decreased the expression of the proapoptotic protein Bax (487). The Bcl-2 family of proteins is divided into three groups, based on structural and functional similarities. Members from group I, such as Bcl-2 and Bcl-X_L, have antiapoptotic roles. Members in groups II and III, such as Bax and Bcl-X_S, play an important role in promoting apoptosis (183).

Burniston and colleagues (62, 64) have reported that (similar to that in the heart) β -adrenoceptor stimulation in skeletal muscle also results in a low level of apoptosis, more so in slow- than in fast-twitch skeletal muscle. In contrast to β_1 -adrenoceptor-mediated apoptosis in cardiomyocytes, the β_2 -adrenoceptor was thought responsible for the skeletal muscle apoptosis (62, 64).

J. Regulation of β -Adrenoceptor Expression and Function

β -Adrenoceptor function is maintained via an equilibrium in processes that mediate receptor density, including synthesis and downregulation (352). While these processes alter adrenoceptor function over an extended period of time, adrenoceptor function can be adjusted immediately via mechanisms that modify receptor sensitivity, including receptor sensitization, phosphorylation, and internalization (94, 352).

1. β -Adrenoceptor phosphorylation

Continuous activation of β -adrenoceptors initiates pathways that lead to a rapid attenuation of the biological response, a process known as receptor desensitization (316). The major mechanism of β -adrenoceptor desensitization involves receptor phosphorylation, which occurs via the actions of protein kinases (such as PKA and PKC) as well as tyrosine kinases (such as the GPCR kinases, termed "GRK"). β -Adrenoceptor phosphorylation can be specific to those receptors that have been activated (a process termed homologous desensitization, Fig. 5A), or nonspecific and involve quiescent receptors (known as heterologous desensitization, Fig. 5B) (92, 299, 316, 369).

Heterologous desensitization occurs rapidly following β -adrenoceptor activation and involves the PKA-mediated nonspecific phosphorylation of both active and

inactive β -adrenoceptors (207, 257). PKA is believed to phosphorylate serine²⁶² in the third intracellular trans-membrane loop of the β_2 -adrenoceptor, rapidly reducing the affinity of the receptor for G protein binding (202, 443). In contrast, homologous desensitization occurs when GRK phosphorylates the β_2 -adrenoceptor in an occupancy-dependent manner and therefore only receptors that have been activated are phosphorylated. GRK phosphorylation occurs mainly within the COOH terminal of the β_2 -adrenoceptor, at serine residues 355 and 356 (443).

The GRK family consists of six known isoforms, GRK1–6, and includes the rhodopsin kinase subfamily (GRK1), two β -adrenoceptor kinases (GRK2, also referred to as β -adrenoceptor kinase or β ARK, and GRK3) and GRK4, GRK5, and GRK6 (200, 363). Rat skeletal muscle contains predominantly GRK2 and GRK5, with a greater overall expression of GRK protein in fast- than in slow-twitch skeletal muscle (209).

The phosphorylation of β -adrenoceptors by GRK2 targets the COOH terminal of the adrenoceptor for binding by a member of the arrestin protein family (314, 362). Binding of arrestin to the adrenoceptor uncouples the receptor from G protein binding and targets the receptor to clathrin-coated pits for internalization and subsequent recycling to the membrane or degradation (238, 314).

While GRK2-mediated β -adrenoceptor phosphorylation is initiated via receptor activation, Whalen et al. (459) demonstrated that GRK2 activity was regulated by low-molecular-weight *S*-nitrosothiols (SNOs). *S*-Nitrosylation of GRK2 reduced phosphorylation and internalization of the β -adrenoceptor in HEK293 cells and prolonged the cAMP signaling response to receptor activation (459). Therefore, GRK2 activity and subsequent β -adrenoceptor phosphorylation is both positively and negatively regulated.

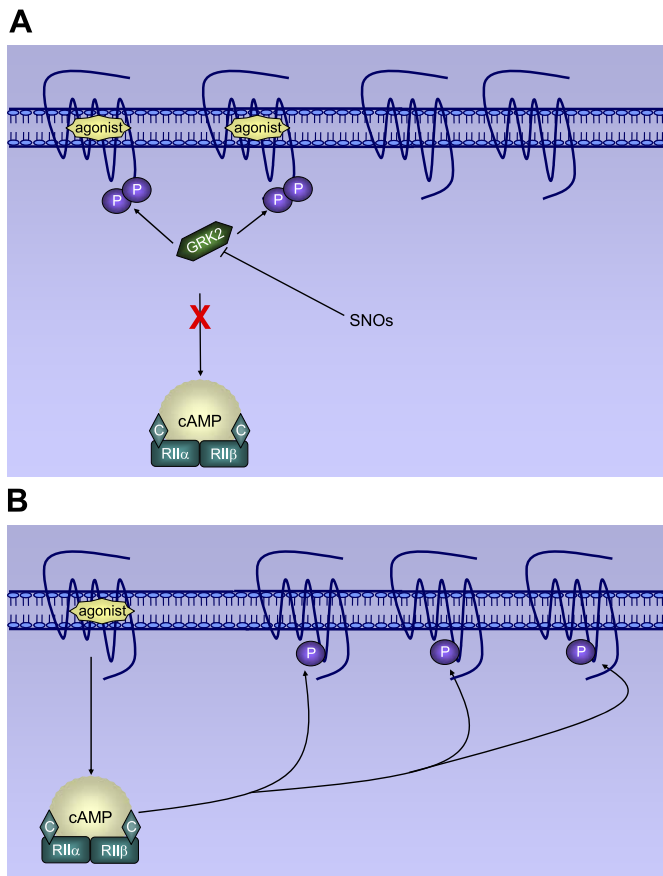


FIG. 5. Mechanisms of β -adrenoceptor phosphorylation. A: the COOH terminus of agonist-bound β -adrenoceptors is targeted for phosphorylation predominantly at residues Ser³⁵⁵ and Ser³⁵⁶ by GRK2 (also termed β ARK). As GRK2 does not phosphorylate unbound β -adrenoceptors, this process of phosphorylation is described as homologous desensitization (443). GRK-mediated homologous desensitization can be inhibited by the nitrosylation of GRK2 by SNOs (459). B: heterologous desensitization involves the PKA-mediated phosphorylation of Ser²⁶² in the third intracellular loop of the β -adrenoceptor. PKA indiscriminately phosphorylates both agonist-bound and -unbound β -adrenoceptors in a process termed heterologous desensitization (202, 207).

2. β -Adrenoceptor internalization and downregulation

Following β -adrenoceptor phosphorylation, the receptor is internalized for dephosphorylation and subsequent recycling to the membrane surface, or for degradation (114, 314, 401). β -Adrenoceptors are generally believed to be internalized via the formation of clathrin-coated pits, although other studies have also identified a clathrin-independent mechanism of internalization (369).

The classic mechanism of β -adrenoceptor internalization involves GRK2-mediated phosphorylation and subsequent binding of an arrestin protein. 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) (166). The nonvisual arrestins, β -arrestin and β -arrestin2, are expressed ubiquitously in all cells and tissues and function to terminate the signals of many GPCRs and

initiate processes leading to receptor internalization (114).

β -Adrenoceptor internalization is mediated primarily via β -arrestin2, which acts as an adapter protein for β 2-adaptin (AP2) and clathrin (154, 251). Following β -adrenoceptor activation and requisite GRK2 receptor phosphorylation, cytoplasmic β -arrestin2 is activated via dephosphorylation by casein kinase II (CK2) and weakly binds to the COOH terminal of the β -adrenoceptor (224, 237, 258). Following the targeting of the β -adrenoceptor to clathrin-coated pits, the weakly bound β -arrestin2 is released and rephosphorylated. β -Adrenoceptor internalization is followed by sorting to recycling endosomes, which traffic receptors back to the cell surface, or to multivesicular late endosomes which traffic receptors for degradation (Fig. 6) (314).

Rapacciuolo et al. (369) examined the role of PKA phosphorylation in HEK293 cells transfected with one of three β_1 -adrenoceptor mutations in the PKA phosphorylation region (PKA^-), the GRK phosphorylation region (GRK^-), or both ($\text{PKA}^-/\text{GRK}^-$). Following stimulation with a β_1 -agonist, both the PKA^- and the GRK^- mutants exhibited similar levels of β_1 -adrenoceptor internalization. However, following the addition of clathrin inhibitors, only the PKA^- mutant cells demonstrated measurable levels of β_1 -adrenoceptor internalization. Interestingly, the addition of caveolin inhibitors prevented β_1 -adrenoceptor internalization in GRK^- cells, indicating a caveolae-dependent

mechanism of internalization following PKA phosphorylation. These results indicate that GRK2-induced internalization is clathrin mediated, while PKA-induced internalization occurs through caveolae. Whether similar independent PKA and GRK2 internalization mechanisms exist for the β_2 -adrenoceptor has yet to be determined.

While the processes of receptor internalization, recycling, and degradation have generated intense interest, the molecular mechanisms responsible for these processes are not well understood. β -Arrestin function is regulated by ubiquitination, in addition to the well-described processes of phosphorylation and dephosphorylation. Ubiquitin, while traditionally associated with marking proteins for destruction, is now understood to also play a role in protein trafficking and signal transduction (114, 457, 463).

Shenoy et al. (402) demonstrated that following activation of the β -adrenoceptor in COS-7 cells, both the receptor and β -arrestin were ubiquitinated by the E3 ligase Mdm2. Interestingly, in Mdm2-null cells, β -adrenoceptor internalization was impaired, but receptor degradation was unaffected. However, when a β -adrenoceptor mutant incapable of ubiquitination was expressed, the receptor was internalized but degradation was impaired. These results indicate that Mdm2 ubiquitination of β -arrestin2 is important for β -adrenoceptor internalization, but not for degradation, and that β -adrenoceptor ubiquitination is required for degradation, but is mediated via a different, as yet undefined, E3 ligase (402).

The ability of β -adrenoceptors (and all GPCRs) to downregulate following chronic activation is important for the maintenance of intracellular homeostasis. However, under certain conditions, it is advantageous to inhibit these homeostatic mechanisms and thus maximize the β -adrenoceptor signal (17, 18). In contrast, the dysregulation of these homeostatic mechanisms can result in severe pathological changes in some tissues, the best described being cardiac dysfunction associated with increased GRK activity (89, 253, 362).

The mechanisms controlling the β -adrenoceptor signaling pathway in many tissues can be altered under certain conditions. For example, Auman and colleagues (17, 18) demonstrated that during fetal and neonatal development, cardiac β -adrenoceptors are resistant to β -adrenoceptor desensitization. This finding was particularly important since catecholamine levels are increased significantly in the neonate and would be expected to cause significant desensitization during this period (245). The underlying mechanism(s) and physiological benefit derived from the lack of desensitization has yet to be fully described.

In a study by Morton et al. (317), β -adrenoceptor stimulation in neonatal rats from postpartum day 3 until day 15 resulted in an increased body mass and increased mass of soleus and EDL muscles, compared with untreated rats. These results indicate that skeletal muscle

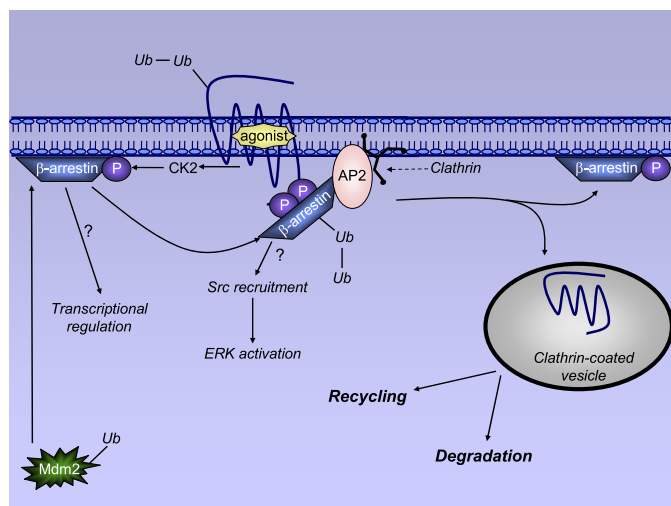


FIG. 6. Role of β -arrestin in β -adrenoceptor internalization, recycling, and degradation. Following phosphorylation of the β -adrenoceptor by GRK2, the COOH terminus is targeted for binding by β -arrestin (314). β -Arrestin binding uncouples β -adrenoceptor-G protein binding and is involved in the movement of the receptor to clathrin-coated pits for internalization (regulated via Mdm2-mediated β -arrestin ubiquitination) and subsequent recycling or degradation (402). In addition to its role in internalization, β -arrestin has been found to be involved in β -adrenoceptor ubiquitination (through an as yet unidentified E3 ligase), signal transduction, possibly via activation of the ERK signaling pathway, and activation of regulators of transcription (114, 457, 463).

β -adrenoceptors are present in the neonate and involved in muscle growth. To our knowledge, no studies have examined the role of the β -adrenergic signaling pathway in the developmental regulation of skeletal muscle, or whether a lack of desensitization (similar to that in the heart) is observed (17, 18).

Studies from our laboratory have determined that β -adrenoceptor downregulation in rat skeletal and cardiac muscle is altered with age. Following chronic β -adrenoceptor activation, we observed a reduced level of receptor downregulation in fast-twitch skeletal muscles from old compared with young rats, whereas in cardiac muscle we observed a greater level of downregulation in old compared with young rats (160, 387).

In another study, Larkin et al. (252) found that β -adrenoceptor density was increased in the gastrocnemius muscles of old compared with young rats. These results were in contrast to the finding that β -adrenoceptor density in the heart was decreased with age. Interestingly, the increase in gastrocnemius muscle β -adrenoceptor density in old rats was associated with an increased basal activity of AC. These results suggest that the β -adrenergic signaling pathway is preserved in the predominantly fast-twitch gastrocnemius muscle (252).

The results of Larkin et al. (252) were supported by findings from our laboratory of a similar hypertrophic response to β -adrenoceptor stimulation in the fast-twitch EDL muscles from young, adult, and old rats (388). However, in the same study we demonstrated that the response of the slow-twitch soleus muscle to β -adrenoceptor stimulation was reduced significantly. These results suggested that the processes regulating β -adrenoceptor downregulation in skeletal and cardiac muscle were altered with aging in the rat and that the processes regulating downregulation were tissue specific (252, 358, 388).

3. β -Adrenoceptor synthesis

Following β -adrenoceptor downregulation, transcription and subsequent translation of the β -adrenoceptor gene is required to restore transmembrane receptor number (207). While there is a wealth of information regarding β -adrenoceptor downregulation (94, 352), our knowledge regarding the molecular regulators of β -adrenoceptor synthesis is lacking.

A number of studies have demonstrated that hormones that act on nuclear receptors can increase the abundance of β -adrenoceptors. In particular, administration of glucocorticoids or thyroid hormone, either in vitro or in vivo, can result in a significant increase in β -adrenoceptor density and mRNA levels in smooth and cardiac muscle, and in adipose tissue (20, 37, 163, 168, 177, 244). The precise molecular mechanism for the regulation of β -adrenoceptor density in these tissues has yet to be determined. However, research focused on the glucocor-

ticoid-induced increase in β -adrenoceptor mRNA has identified a glucocorticoid-response element (GRE) in the mammalian β_1 - and β_2 -adrenoceptor genes, which likely acts to increase mRNA transcription at these sites (168, 276, 316).

In animal studies focused on skeletal muscle, glucocorticoid treatment has not been associated with an increase in adrenoceptor density. Two studies by Huang and colleagues (192, 193) demonstrated that administration of glucocorticoids to rats for 5 or 10 days increased β_2 -adrenoceptor density in lung tissue, but had no effect on skeletal muscle. These results further supported the hypothesis that the regulation of β -adrenoceptor synthesis was tissue specific.

Interestingly, the activation of β -adrenoceptors themselves may act in a feedforward manner to increase β -adrenoceptor mRNA transcription via cAMP-mediated CRE activation. In cell culture models, short-term activation of β -adrenoceptors results in a three- to fivefold increase in receptor mRNA levels (96, 97).

Under certain conditions, skeletal muscle β -adrenoceptor density can increase. Our laboratory has shown that in EDL muscles of the rat injured by injection of the myotoxin bupivacaine hydrochloride, there was a two- to threefold increase in β -adrenoceptor density (Fig. 7). The mechanism for this increase in skeletal muscle adrenoceptor density remains unclear but would likely result in an increased response to circulating catecholamines and an increased rate of regeneration (31).

IV. β -ADRENOCEPTOR AGONISTS

Although traditionally used for the treatment of bronchial ailments, especially asthma, it quickly became apparent that some β -adrenoceptor agonists (β -agonists) had the ability to increase skeletal muscle mass and decrease body fat, i.e., the so-called "repartitioning effect" (129). As a consequence of their potent muscle anabolic actions, the effects of β -agonist administration have been examined in a number of animal models (and in humans) in the hope of discovering a new therapeutic strategy for muscle wasting disorders (discussed in detail in sect. v) (74, 228, 269, 279, 388). In addition, the combination of muscle hypertrophy and decreased body fat proved desirable for those working in the livestock industry with the aim of improving feed efficiency and meat quality (406). Not surprisingly, β -agonists were soon being used by those engaged in competitive bodybuilding and soon after by other athletes competing in strength- and power-related sports (265, 361). Thus a discussion of the potential beneficial effects of β -agonists on skeletal muscle must be balanced by reviewing some of the (less well reported) deleterious effects of β -agonists on striated muscle (discussed in sect. vi).

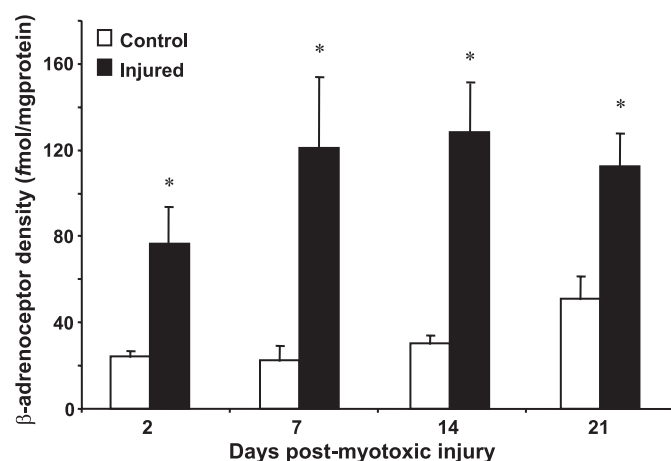


FIG. 7. β -Adrenoceptor density in regenerating rat extensor digitorum longus (EDL) muscle 2, 7, 14, and 21 days postinjury. EDL muscles were injured with the myotoxin bupivacaine hydrochloride. Within 2 days postinjury, the EDL β -adrenoceptor density was increased twofold and remained elevated for the 21 days examined. These significant findings highlight the potentially critical role of the β -adrenergic signaling pathway in successful skeletal muscle regeneration. [Adapted from Beitzel et al. (31).]

The chemical structure of adrenaline (epinephrine) and a selection of β -agonists are shown in Figure 8. Common β -agonists include salbutamol (albuterol), bambuterol, terbutaline, fenoterol, mapenterol, formoterol, tulobuterol, carbuterol, bromobuterol, cimbuterol, zinterol, cimaterol, ractopamine, mabuterol, salmeterol, and clenbuterol. With respect to our understanding of the effects of β -agonists on skeletal and cardiac muscle, there is a wealth of data on the effects of clenbuterol, more so than other β -agonists (see Table 2).

Clenbuterol [1-(4-amino-3,5-dichlorophenyl)-2-ter-butylaminoethanol] is defined as a sympathomimetic amine, i.e., its actions mimic that of adrenaline (epinephrine). Clenbuterol, like most β -agonists, is used primarily for the treatment of asthma and related bronchospasm (279). It is a powerful bronchodilator for human patients, but it also has widespread veterinary applications in the equine and livestock industries (361). Brand names for the generic name clenbuterol include the following: Clenasma, Monores, Novegam, Prontovent, Spiropent, Broncoterol, Broncodil, Cesbron, Clenbuter, Pharmachim, Contrasma, Contraspasma, Monores, Oxyflux, Ventolase, Ventipulmin, and Clenbumar. It is available in tablet form in 10 and 20 μ g doses and can be obtained as a powder for use in making solutions of varying concentration. The dose of clenbuterol for use in puffers (inhalers) for the treatment of asthma in humans ranges between 0.02 and 0.03 mg twice daily (361). With infrequent use and at such low dosages, asthmatics experience few side effects of clenbuterol administration. Surprisingly, clenbuterol is not approved for use by humans as a bronchodilator in the United States nor does it have

Federal Drug Administration approval. Albuterol (salbutamol) is listed as the alternative drug to clenbuterol for use by asthmatics (361). An orally administered syrup containing clenbuterol (Ventipulmin) is available in the United States for use in horses affected with airway obstruction, such as that associated with chronic obstructive pulmonary disease (COPD). In other countries, including Europe, Australia, Canada, and South America, Ventipulmin has been available for more than 10 years. The duration of clenbuterol treatment recommended for horses is 30 days (at a dose of 0.8 μ g/kg). Clenbuterol is currently approved in the United Kingdom for the treatment of asthma in human patients.

A. Acute Response to β -Adrenoceptor Agonist Stimulation

Although the primary focus of this review is on the effects of chronic β -agonist administration on skeletal muscle, it is also important to highlight some of the acute responses. Much of our understanding of the acute effects of β -agonist administration on skeletal muscle has come from the multitude of studies that have examined chronic and acute administration of adrenaline or related sympathomimetic agents. Among the most significant contributions to our understanding of the effects of adrenergic activators and inhibitors on skeletal muscle are the reviews of Prof. William Bowman and colleagues (54, 55). In addition to characterizing the effects of adrenaline on skeletal muscle, many of the unwanted effects of β -adrenergic stimulation were in these reviews, including muscle tremor (54, 55).

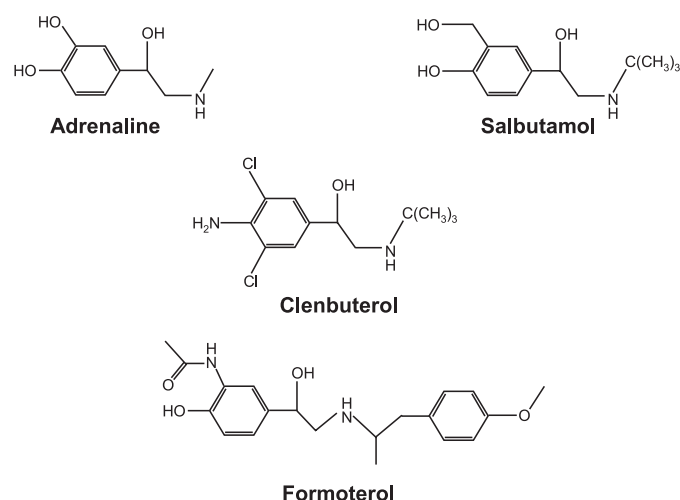


FIG. 8. Chemical structure of adrenaline (epinephrine) and a number of common synthetic β_2 -agonists. A single benzene ring is common to all β_2 -agonists; however, new generation β_2 -agonists such as formoterol (and salmeterol) are characterized by the addition of a long carbon chain containing a second benzene ring, which is likely responsible for their long duration of action at the β -adrenoceptor (27, 453).

Acute adrenaline administration increases force production in fast muscle but reduces it in slow muscles. The mechanism of action by which adrenaline (or sympathomimetic amines) exerts these differential effects on fast and slow muscles has been a long-standing issue in pharmacology (167). There are significant differences between fast and slow muscles with respect to adrenoceptor density (higher in fast than in slow), Ca^{2+} -handling characteristics, and regulatory protein composition, and these and other factors may contribute to the differential response to acute adrenaline administration. For example, the positive inotropic effect on fast muscles (observed in a variety of animal species) was thought to be due to cAMP-enhanced Ca^{2+} exchange within the muscle fiber and/or to increased influx of extracellular Ca^{2+} , consistent with the mechanism of the positive inotropic effects of epinephrine on cardiac muscle (461). In cats, Cairns and Dulhunty (68) examined the effects of β -adrenoceptor activation on twitch and tetanic contractions in bundles of intact fibers from fast- and slow-twitch rat skeletal muscle fibers in vitro. Addition of the β -agonist terbutaline to the bathing medium potentiated peak twitch and tetanic force in soleus muscle fibers and hastened the rate of twitch and tetanic relaxation. In later experiments, the effects of terbutaline on force production in rat soleus muscle fibers were explained by changes in the amount of SR Ca^{2+} released and the speed of SR Ca^{2+} reuptake, and not to changes in the Ca^{2+} sensitivity or force-generating capacity of the contractile proteins (167).

B. Effects of Chronic β -Adrenoceptor Agonist Administration: Evidence From Animal Studies

1. Growth-promoting effects of β -agonists

The use of β -agonists in the livestock industry revealed a number of interesting side effects, namely, that in high doses, β_2 -agonist administration produced an increase in skeletal muscle mass and a concomitant decrease in body fat (30, 34, 171, 188, 194, 235, 305, 308, 372, 377, 396). As such, β_2 -agonists such as clenbuterol (and cimaterol) became known as a "repartitioning agents" (223, 304). These studies in a number of animal species have demonstrated that when administered in high doses (i.e., higher than what could be tolerated by humans), extended use of β -agonists can elicit significant increases in muscle mass (6, 22, 26, 30, 74, 78, 90, 113, 129, 164, 225, 270, 281, 297, 344, 372, 377, 383, 386, 408, 440, 456, 474, 478, 479), in the order of 10–25% after 10–20 days of administration. The increase in muscle protein is considered "true" muscle hypertrophy (474) since hyperplasia (increased cell number) and satellite cell division are not associated with the protein increases (278). Other β -agonists that have proven effective in producing a muscle hypertrophic response include cimaterol, salbutamol, iso-

proterenol, and ractopamine, although clenbuterol ranks as one of the more potent of these compounds when administered at high (mg/kg) doses.

That β_2 -adrenoceptors mediate the anabolic effects of clenbuterol was originally confirmed by the actions of the selective antagonist ICI118551, which has an ~ 100 -fold greater affinity for β_2 -adrenoceptors than for β_1 -adrenoceptors. ICI118551 reduced the anabolic effects of clenbuterol, and when administered alone caused muscle atrophy (408). Further confirmation was provided by Hinkle et al. (187), who administered clenbuterol to control, β_1 -, or β_2 -adrenoceptor knockout mice and demonstrated that only control and β_1 -adrenoceptor knockout mice exhibited skeletal muscle hypertrophy. These studies confirmed that β_2 -adrenoceptors mediated the pharmacological effects of clenbuterol and that they were involved in the control of muscle growth.

The basis for the effectiveness of clenbuterol as an anabolic agent has been attributed to its ability to promote muscle protein synthesis (90, 93, 129, 201, 281, 372, 377) as well as reduce muscle protein degradation (39, 51, 139, 278, 281, 372, 454). There has been much debate as to whether the clenbuterol-induced increase in muscle mass is due preferentially to one mechanism or the other (34). Early evidence from studies on livestock attributed the anabolic properties primarily to the ability of clenbuterol to inhibit protein degradation (407). More recent studies, however, indicate that these effects are also driven by activation of the Akt/mTOR pathway driving protein translation (230), highlighting the fact that clenbuterol activates pathways responsible for attenuating protein degradation and promoting protein synthesis.

Kline et al. (230) showed that coadministration of rapamycin and clenbuterol produced different responses in atrophying skeletal muscles during denervation or hindlimb suspension. Rapamycin treatment inhibited the anabolic effect of clenbuterol in unloaded fast muscles but not in denervated fast muscles, findings attributed to the different mechanisms underlying denervation and hindlimb unloading. Although both interventions caused increased protein degradation and decreased protein synthesis, the authors argued that suppressing protein synthesis would have greater significance for hindlimb suspension than denervation because the Akt/mTOR signaling pathway is inactivated with unloading (50). Their studies revealed that clenbuterol activated the Akt/mTOR pathway and increased protein translation such that the muscle loss concomitant with unloading was suppressed. Their confirmation of rapamycin inhibition of clenbuterol's effects indicated that activating pathways downstream of mTOR was responsible for attenuating the loss of muscle mass during unloading (230).

Anecdotal reports indicate that the muscle anabolic effects of clenbuterol in humans are much less pronounced than those observed in livestock. In fact, studies

on cattle, sheep, and pigs have shown that the mechanism controlling tissue responsiveness to β -agonists varies from species to species, and even among different tissues within a species, primarily because of differences in the densities of each of the receptor subtypes (185, 407). Considerable knowledge about the effects of β -agonists on skeletal muscle mass has come from the many studies examining the use of these compounds in livestock, especially with respect to their potential to improve meat quantity and to a lesser extent, quality (29, 30, 34, 93, 165, 235, 304, 306, 373, 398). For a comprehensive analysis of technologies for controlling fat and lean deposition in livestock, including the use of β -agonists, the reader is referred to the review of Sillence (406).

β -Agonist administration has been shown to produce hypertrophy of both fast- and slow-twitch muscle fibers in rats and mice (11). The literature describing these effects caused by chronic administration of β -agonists such as clenbuterol has been divided as to whether the effects were greater in type II (fast-twitch) muscle fibers (30, 278, 308, 344, 479), in type I (slow-twitch) muscle fibers (30, 278, 344), or similar in both types (373, 400). However, it should be noted that the magnitude of the anabolic response of skeletal muscles to β -agonist administration is likely dependent on several factors such as the β -agonist employed, the mode of administration, the dose and frequency of administration, the duration of treatment, the species used, and the age of the animal at the time of treatment.

Older generation β -agonists, such as clenbuterol or fenoterol, are powerful muscle anabolic agents when administered to rats at relatively high (mg/kg) doses but elicit a markedly lesser effect when administered at what would be considered therapeutic doses (μ g/kg), such as the doses employed by human (asthmatic) patients and other species (e.g., horses) for the management of inflammatory airway disease (277, 357). Chen and Alway (85) found that clenbuterol administered to rats at a low dose of $10 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ had only modest effects on slow-twitch skeletal muscle and no discernable effect on fast-twitch skeletal muscles. In contrast, newer generation β -agonists such as formoterol and salmeterol when administered to rats daily via intraperitoneal injection at very low doses (μ g/kg) can still elicit significant anabolic responses in fast and slow muscles of the rat (389). A study from our laboratory indicates that formoterol administration to rats at a dose of only $1 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ can still elicit muscle hypertrophy in both fast- and slow-twitch skeletal muscles (389).

When comparing the effects of β -agonists on skeletal muscle size and strength in different animal models, it is also important to consider the mode of β -agonist administration, i.e., whether administered orally via the drinking water (70, 181, 337, 351, 376, 423, 482), via the feed (76), via a slow-release pellet (85), oral gavage (57), mini-os-

motric pump (74), or via intraperitoneal or subcutaneous injection (21, 117, 208, 209, 367, 411). While administration of β -agonists via the food or drinking water is convenient, there is uncertainty as to whether every treated animal will receive an identical dose. Thus it is preferable to utilize a mode of administration where an exact dose can be administered. β -Agonist administration to animals, whether via drinking water, oral gavage (or ingestion), or systemic administration (39, 315, 388, 411), is associated with an initial drop of 5–10% of body mass in the first 2–5 days of treatment. This response is believed to be due to stimulation of central β_2 -adrenoceptors in the hypothalamus which causes a transitory suppression of appetite (35).

Clearly, the potency of some β -agonists (for eliciting an anabolic response in skeletal muscle) is dependent on the route of administration (315). When administered to rats orally in their drinking water for 10 days, low-dose salmeterol treatment did not produce an anabolic response in skeletal muscles (315). However, when administered by intraperitoneal injection, daily low-dose salmeterol did produce a significant muscle anabolic response (389). When administered to rats via osmotic minipump, clenbuterol and salmeterol caused significant increases in muscle mass, whereas clenbuterol was more potent than salmeterol when administered orally (315).

2. β -Agonist-induced muscle fiber transitions

Numerous studies have reported that administration of β -agonists (such as clenbuterol, fenoterol, and formoterol) to rats and mice can produce slow-to-fast muscle fiber transitions, especially within the predominantly slow-twitch soleus muscle (6, 30, 181, 278, 344, 480). Fiber transitions can also occur within the subtypes of the fast fiber populations, such as the conversion of fast oxidative glycolytic (type IIA) fibers towards those having a more glycolytic metabolism (i.e., fast glycolytic or type IIB muscle fibers) (278). In small mammals, chronic treatment with β -agonists like clenbuterol has caused typically slow muscles to become more “fastlike” with respect to their fiber type composition, not just their biochemical make up (oxidative to glycolytic), but also in their myosin heavy chain (MHC) isoform composition, and hence their contractile properties (268). Similar fiber type transitions within the skeletal muscles of animals are usually only observed during muscle development (105), following chronic high-frequency electrical stimulation (348), denervation, hormonal manipulation (67), unloading, muscle regeneration (159), and to a very limited extent following exercise (158, 273).

The molecular mechanisms controlling the slow-twitch muscle fiber phenotype have received a great deal of attention and include activation of calcineurin and NFAT, CaMK, peroxisome proliferator-activated receptor

gamma coactivator 1 (PGC1 α), peroxisome proliferator-activated receptor δ (PPAR δ), and Ras (87, 259, 264, 300, 321, 330). However, our understanding of the molecular mechanisms controlling the expression of the fast-twitch muscle fiber phenotype is less well established.

The first evidence of a transcriptional pathway controlling the expression of the fast-twitch glycolytic phenotype was provided by Grifone et al. (162). In this study, Six1, when bound with the cofactor Eya1, was found to exert transcriptional regulation over the expression of fast-twitch MHC proteins. The Six1/Eya1 complex induced the expression of fast MHC proteins via binding to MEF3 control elements. Interestingly, when Six1 and Eya1 plasmids were cotransfected via electroporation into the soleus muscles of mice, there was a shift in fiber phenotype from the slow type I/IIA to the fast type IIB (162), a response similar to that observed with β -agonist administration. To date, no study has examined whether β -agonist administration alters the levels of Six1 and Eya1 in skeletal muscle.

3. Thermogenesis

Studies in both humans and animals have shown that β -agonists such as clenbuterol have powerful lipolytic effects due to its thermogenic (heat-producing) properties (12, 13, 33, 41, 47, 275, 372, 383, 384). Adipose tissue is a major site for thermogenesis. White adipose tissue is a major site for fat storage, and β -agonists act on the adrenoceptors in this tissue to increase lipolysis (310). β -Agonists, such as clenbuterol, stimulate the breakdown of fat and increase energy expenditure. Brown adipose tissue is almost nonexistent in humans soon after birth, whereas other mammals have greater brown adipose stores. The β_3 -adrenoceptors are primarily involved with brown adipose tissue.

V. THERAPEUTIC POTENTIAL OF β -AGONISTS: IMPLICATIONS FOR MUSCLE-WASTING DISORDERS

There have been numerous studies on animals and several studies on humans regarding the effects of β -agonists on skeletal muscle. A selection of these studies and their main findings are presented in Table 2. Muscle wasting and weakness are common in many disease states and conditions, including aging, cancer cachexia, sepsis or other forms of catabolic stress, denervation, disuse, inactivity, burns, HIV-acquired immunodeficiency syndrome (AIDS), chronic kidney or heart failure, COPD, unloading or microgravity, and the muscular dystrophies. For many of these conditions, the anabolic properties of β -agonists provide therapeutic potential for attenuating or potentially reversing the muscle wasting, muscle fiber atrophy, and associated muscle weakness. Certainly, at high doses,

β -agonists such as clenbuterol have been shown to preserve muscle mass and function during disuse. The anabolic properties of β -agonists also have important clinical significance for enhancing muscle repair and restoring muscle function after muscle injury or following reconstructive surgery (31).

A. Age-Related Muscle Wasting and Weakness

“Sarcopenia” is the term widely used to describe the slow, progressive loss of muscle mass with advancing age. The underlying mechanisms of age-related muscle wasting and weakness and potential therapeutic approaches for sarcopenia have been described in detail elsewhere (272). One approach for consideration is the use of anabolic agents such as β -agonists (266, 267) to attenuate the loss of muscle fiber size, the loss of muscle strength, and the potential remodeling of muscle due to the eventual loss of fast muscle fibers that compromise functional capacity and the performance of the tasks of everyday living (197).

In relation to attenuating the loss of muscle mass and protein content or hastening the restoration of these parameters in the elderly during periods of malnutrition or extended periods of inactivity, three early studies by Carter and Lynch (75–77) provided encouraging evidence that β -agonists could find therapeutic application for these conditions. To examine the anabolic effects of low-dose salbutamol or clenbuterol administration on aged rats, Carter and Lynch (75) showed that in old rats, subcutaneous delivery by osmotic minipumps (at daily doses of 1.03 mg/kg or 600 μ g/kg) for 3 wk increased combined hindlimb muscle mass by 19 and 25%, respectively. Gastrocnemius muscle mass and protein content were increased by 19 and 23%, respectively, in old rats. Salbutamol and clenbuterol increased skeletal muscle protein content and reduced carcass fat content, findings that suggested both β -agonists could potentially stimulate muscle growth in frail elders (75).

In a related experiment, Carter and Lynch (76) studied the effect of clenbuterol on recovery of muscle mass and carcass protein content after protein malnutrition in aged rats. The rats were subjected to 3 wk of dietary protein restriction that reduced overall body mass by 21%. During the recovery period, the rats were fed a normal diet with clenbuterol (10 mg/kg) added to the feed. The addition of clenbuterol to the diet increased hindlimb muscle mass by 30% and protein content by 25% in aged rats (76). In another experiment (77), aged rats were injected daily with thyroid hormone (4–6.5 mg triiodothyronine per 100 g body mass) for 3 wk to cause an \sim 20% reduction in body mass and hindlimb muscle mass. Feeding the rats a diet containing 10 mg/kg clenbuterol during a 3-wk recovery period restored body mass and muscle

TABLE 2. *Selected studies that have examined the therapeutic potential of β -agonists to prevent or reverse muscle wasting in a number of pathological conditions where muscle wasting is indicated*

Muscle Wasting Condition	β_2 -Agonist Administered	Route of Administration	Animal Model	Main Findings	Reference No.
Aging	Clenbuterol (10 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 21 days)	sc infusion	Fischer 344 \times Brown Norway rat	\uparrow Soleus mass, \uparrow soleus strength, \leftrightarrow soleus muscle mass normalized to BM, \leftrightarrow Plantaris mass, \leftrightarrow plantaris strength	85
	Clenbuterol (10 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 21 days)	sc infusion	Fischer 344 \times Brown Norway rat	\uparrow Soleus strength, \downarrow soleus fatigability, \leftrightarrow plantaris strength, \leftrightarrow plantaris fatigability	84
	Clenbuterol (1.5 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 3 wk)	sc infusion	Fischer 344 rat	\uparrow Muscle mass, \uparrow carcass protein content, \downarrow carcass fat content	74
	Clenbuterol (600 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 3 wk)	sc infusion	Fischer 344 rat	\uparrow Combined hindlimb muscle mass, \uparrow gastrocnemius mass and protein content, \uparrow carcass protein content	75
	Clenbuterol (10 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 3 wk)	Oral (in food)	Fischer 344 rat	\uparrow Combined hindlimb muscle mass, \uparrow carcass protein content	76
	Fenoterol (1.4 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 4 wk)	ip injection	Fischer 344 rat	\uparrow EDL mass, \uparrow EDL strength, \uparrow EDL fatigability, \leftrightarrow EDL fiber-type proportions, \uparrow soleus mass, \uparrow soleus strength, \uparrow soleus fatigability, \uparrow proportion of fast-type II fibers in soleus, \uparrow HM	387
	Fenoterol (1.4 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 4 wk)	ip injection	Fischer 344 rat	\uparrow V_{max} of SERCA activity in gastrocnemius muscle, \uparrow SERCA1 protein levels in gastrocnemius muscle, \downarrow red gastrocnemius SERCA2a nitrosylation	395
	Formoterol (25 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 4 wk)	ip injection	Fischer 344 rat	\uparrow BM, \uparrow EDL mass, \uparrow EDL strength, \uparrow proportion of fast-type II fibers in EDL, \leftrightarrow EDL fatigability, \leftrightarrow soleus mass, \uparrow soleus strength, \leftrightarrow soleus fatigability, \leftrightarrow proportion of fast-type II fibers in soleus	388
	Salbutamol (1.03 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 3 wk)	sc infusion	Fischer 344 rat	\uparrow Combined hindlimb muscle mass, \uparrow gastrocnemius mass and protein content, \uparrow Quadriceps strength	75
Muscular dystrophy	Albuterol (salbutamol) (8 mg/day for 12 wk)	Oral (tablet)	Human	\uparrow Lean body mass, \uparrow grip strength	140
	Albuterol (salbutamol) (16–32 mg/day for 12 mo)	Oral (tablet)	Human	\uparrow Elbow flexor strength, \downarrow Ankle dorsiflexor strength	227
	Albuterol (salbutamol) (32 mg/day for 12 mo)	Oral (tablet)	Human	\uparrow Lean body mass, \uparrow strength (evaluated through maximal voluntary isometric contraction testing and manual muscle testing)	448
	Albuterol (salbutamol) (16 mg/day for 12 mo)	Oral (tablet)	Human	\uparrow Lean body mass, \uparrow strength (evaluated through maximal voluntary isometric contraction testing and manual muscle testing)	228
	Clenbuterol (2 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 15 wk)	Oral (in drinking water)	mdx mouse	\uparrow EDL mass, \uparrow soleus mass, \uparrow EDL strength, \uparrow soleus strength	181
	Clenbuterol (2 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 20 wk)	Oral (in drinking water)	mdx mouse	\uparrow Soleus mass, \uparrow EDL absolute power	270
	Clenbuterol (1.5 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 12 mo)	Oral (in drinking water)	mdx mouse	\uparrow Soleus mass, \uparrow soleus strength, \uparrow fatigability in diaphragm and soleus	126
	Clenbuterol (2 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 12 mo)	Oral (in drinking water)	mdx mouse	\leftrightarrow Diaphragm strength, \leftrightarrow diaphragm muscle degeneration and necrosis	269

TABLE 2.—Continued

Muscle Wasting Condition	β_2 -Agonist Administered	Route of Administration	Animal Model	Main Findings	Reference No.
Denervation	Formoterol (25 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 4 wk)	ip injection	mdx mouse	\uparrow EDL mass, \uparrow soleus mass, \leftrightarrow diaphragm thickness, \leftrightarrow HM, \uparrow EDL strength, \uparrow soleus strength	174
	Clenbuterol (2 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 4 days)	Oral (in food)	Rowett hooded listar rat	\uparrow Soleus mass, \uparrow soleus fiber CSA, \uparrow soleus protein content	278
	Clenbuterol (1.6 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 2-3 wk)	Oral (in drinking water)	Wistar rat	\uparrow EDL mass, \uparrow soleus mass, \uparrow EDL strength, \uparrow soleus strength	480
	Clenbuterol (0.9 mg/day for 14 days)	Oral (in drinking water)	Sprague-Dawley rat	\uparrow TA mass, \uparrow soleus mass, \uparrow PA mass,	476
	Clenbuterol (3 mg/kg for 3 days)	Oral (in food)	Rowett hooded listar rat	\uparrow Soleus mass, \uparrow HM, \uparrow in membrane PKC activity in denervated muscle	412
	Clenbuterol (3 mg/kg for 7 days)	Oral (in food)	Rowett hooded listar rat	\uparrow Soleus mass, \uparrow HM, \uparrow in membrane PKC activity in denervated muscle	412
	Clenbuterol (2 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 7 days)	Oral (gavage)	Wistar rats	\uparrow Gastrocnemius muscle mass	7
Unloading/disuse	Isoproterenol (12 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 7 days)	Oral (gavage)	Wistar rats	\uparrow Gastrocnemius muscle mass	7
	Salbutamol (0.6 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 14 days)	ip injection	Wistar rat	\uparrow Soleus fiber CSA, \uparrow proportion type I fibers	415
	Clenbuterol (2 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 12 mo)	Oral (in drinking water)	C57/Bl 10 mouse	\uparrow EDL mass, \uparrow soleus mass, \uparrow TA mass, \uparrow HM	271
	Clenbuterol (0.6 mg/day for 2 wk)	Oral (in drinking water)	Wistar rat	\uparrow Soleus mass, \uparrow proportion of fast (type II) fibers	376
	Clenbuterol (0.6 mg/day for 2 wk)	Oral (in drinking water)	Wistar rat	\uparrow BM, \uparrow gastrocnemius mass, \uparrow soleus mass, \uparrow EDL mass, \uparrow proportion of fast (type II) fibers	423
	Clenbuterol (30 mg/l for 4 wk)	Oral (in drinking water)	Wistar rat	\uparrow Soleus mass, \uparrow HM, \uparrow BM, \uparrow proportion of slow/fast hybrid fibers	338
	Clenbuterol (3.5 $\mu\text{mol/kg/day}$ for 7 days)	ip injection	Wistar rat	\uparrow Gastrocnemius mass, \uparrow soleus mass, \uparrow HM	71
Cancer cachexia	Clenbuterol (1 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 8 days)	sc injection	Wistar rat	Largely prevented the tumor induced protein waste in gastrocnemius and heart muscles by restoring normal rates of protein degradation	100
	Salbutamol (3.5 $\mu\text{mol/kg/day}$ for 7 days)	ip injection	Wistar rat	\uparrow Gastrocnemius mass	71
	Salmeterol (3.5 $\mu\text{mol/kg/day}$ for 7 days)	ip injection	Wistar rat	\uparrow Gastrocnemius mass, \uparrow soleus mass, \uparrow HM	71
	Formoterol (2 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 7 days)	ip injection	Wistar rat	\uparrow Gastrocnemius mass, \uparrow TA mass, \uparrow EDL mass, \uparrow HM	66
	Formoterol (2 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 9 days)	ip injection	Bl/6 mice	\uparrow Gastrocnemius mass, \uparrow TA mass, \uparrow soleus mass, \downarrow rate of muscle apoptosis	66
	Clenbuterol (2 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 4 wk)	Oral (gavage)	Wistar rat	\downarrow Whole body adipose tissue mass, \uparrow proportion of fast (type II) fibers in the soleus, \uparrow soleus MyoD protein levels	57
	Fenoterol (1.4 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ for 4 wk)	ip injection	Sprague-Dawley rat	\uparrow EDL mass, \uparrow EDL fiber CSA, \uparrow EDL strength	31

BM, body mass; CSA, cross-sectional area; EDL, extensor digitorum longus; HM, heart mass; ip, intraperitoneal; PKC, protein kinase C; sc, subcutaneous; SERCA, sarco-endoplasmic reticulum Ca^{2+} -ATPase; TA, tibialis anterior; V_{max} , maximal activity; \uparrow , increase; \downarrow , decrease; \leftrightarrow , no change.

mass to euthyroid control levels, whereas feeding the rats a control diet did not (77). Taken together, these findings suggested that clenbuterol or other β -agonists

could find application in hastening recovery of muscle mass as a consequence of malnutrition in frail, elderly humans (76, 77).

In aged rats, clenbuterol treatment (2 mg/kg) via daily injection for 4 wk restored the age-associated decline in the mass and specific force (i.e., normalized force or force per muscle cross-sectional area) of diaphragm muscle strips (411). A much lower dose of clenbuterol (10 $\mu\text{g/kg}$ per day) attenuated the loss of specific force in the soleus muscle only slightly (i.e., by 8%) and reduced fatigue (in response to repeated stimulation) by $\sim 30\%$ in aged rats, with considerable muscle atrophy having been subjected to 21 days of hindlimb suspension (84). However, low-dose clenbuterol treatment did not reduce the loss of specific force in the soleus of adult rats or in the plantaris muscles of old or adult rats. The study concluded that clenbuterol could reduce muscle fatigue in slow muscles during disuse with some clinical implications for reducing fatigue in muscles of the elderly. Findings from this and a related study (85) indicated that low-dose clenbuterol treatment did not attenuate atrophy of fast muscles and only modestly attenuated the atrophy of slow muscles, making it largely ineffective for preventing muscle wasting from disuse atrophy in aged rats.

In a study from our laboratory (387), old rats were treated daily with a relatively high dose of the β -agonist fenoterol (1.4 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ip) or saline for 4 wk. At 28 mo of age, untreated old F344 rats exhibited a loss of skeletal muscle mass and a decrease in force-producing capacity in both fast and slow muscles. Interestingly, the muscle mass, fiber size, and force-producing capacity of EDL and soleus muscles from old rats treated with fenoterol was equivalent to, or greater than, untreated adult rats (387). Fenoterol treatment caused a small increase in the fatigability of both EDL and soleus muscles due to a decrease in oxidative metabolism. The findings highlighted the clinical potential of β -agonists to increase muscle mass and function to levels that exceeded those in adult rats.

Schertzer et al. (395) found that treating aged rats with fenoterol (1.4 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ip) for 4 wk reversed the slowing of (twitch) relaxation in slow- and fast-twitch skeletal muscle due to increased SERCA activity and SERCA protein levels. The study provided evidence for an age-related alteration in the environment of the nucleotide binding domain and/or a selective nitration of the SERCA2a isoform, which was associated with the depression in SERCA activity. Fenoterol treatment ameliorated the age-related decrease in nucleotide binding affinity and reversed the age-related accumulation of nitrotyrosine residues on the SERCA2a isoform. These changes, in combination with increases in SERCA1 protein levels, appeared to be the underlying mechanisms of fenoterol treatment reversing age-related decreases in the V_{max} of SERCA (395).

In a later study (389), we demonstrated that “newer” generation β -agonists, formoterol and salmeterol, could exert significant anabolic actions on skeletal muscle even

at micromolar doses, compared with the millimolar doses required to elicit the same responses with older generation β -agonists such as fenoterol or clenbuterol. Using this information, we investigated the potential of one of these newer generation β -agonists, formoterol, to increase muscle mass and force-producing capacity of EDL and soleus muscles in aged rats (388). In addition, we studied the effects of formoterol withdrawal on parameters such as muscle mass and strength. Rats were treated with either formoterol (25 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ip) or saline vehicle for 4 wk, and another group of rats was similarly treated with formoterol, followed by a period of withdrawal for 4 wk. Formoterol treatment increased EDL muscle mass and the force-producing capacity of both EDL and soleus muscles, without a concomitant increase in heart mass. The hypertrophy and increased force in EDL muscles persisted 4 wk after treatment withdrawal. This study was important because it demonstrated significant improvements in muscle function in old rats after β -agonist administration, at a dose 1/50 of that of other β -agonists that had been used previously (387). These findings have important implications for clinical trials that might utilize β -agonists for sarcopenia and other muscle-wasting conditions (140, 227, 228).

B. Muscular Dystrophy

There have been numerous studies that have focused on therapeutic applications of the anabolic properties of β -agonists for ameliorating the muscle wasting and weakness relevant to muscular dystrophy (5, 91, 125, 140, 227, 228, 279, 285, 432, 484). Primarily, these studies have utilized the *mdx* mouse, the most commonly used animal model of Duchenne muscular dystrophy (DMD) (126, 174, 179, 181, 269–271, 383, 481), but others have examined the effects of β -agonists, especially clenbuterol, on other murine models of dystrophy, including the laminin-deficient dystrophic mouse (180).

Most of these studies have demonstrated clearly that dystrophic skeletal muscles can respond favorably to β -agonist administration, with most (but not all) studies reporting increases in muscle mass. The effects on force-producing capacity of dystrophic muscles have been less consistent, especially with regard to improving absolute and/or specific force. Harcourt et al. (174) examined whether a low dose (25 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ip) of the newer generation β -agonist formoterol for 4 wk could improve muscle function in *mdx* mice. Low-dose formoterol treatment increased EDL and soleus muscle mass; increased median muscle fiber size in diaphragm, EDL, and soleus muscles; and increased maximum force-producing capacity in skeletal muscles of both wild-type and *mdx* mice. Furthermore, in contrast to other studies where β -agonists have been administered to mice and rats, generally

at higher doses, low-dose formoterol treatment did not increase the fatigability of EDL or soleus muscles, or of diaphragm muscle strips. This is important since muscles from boys with DMD are already highly susceptible to fatigue (414), so any deleterious shift in muscle fiber proportions or metabolism could increase fatigability, reducing the clinical merit of the proposed intervention. These findings indicated that formoterol had considerably more powerful anabolic effects on skeletal muscle than older generation β -agonists (like clenbuterol and albuterol) and had considerable therapeutic potential for muscular dystrophies and other muscular disorders where muscle wasting is indicated.

However, it should be noted that β -agonist administration to dystrophic *mdx* mice has not led to improvements in diaphragm muscle function such as normalized force (or power output), either with clenbuterol or formoterol (174, 270). The diaphragm is the most severely affected muscle in *mdx* mice, so demonstrating improvements in diaphragm function has important clinical implications since respiratory insufficiency is a major hallmark of DMD (234, 289).

Several clinical trials have investigated the potential of the β -agonist albuterol to improve skeletal muscle function in different neuromuscular disorders (140, 227, 228, 340). Preliminary trials using albuterol to treat young boys with facioscapulohumeral dystrophy found that year-long administration at doses of 16 and 32 mg/day had only limited beneficial effects on strength and was associated with some adverse cardiac-related events (228). A Japanese study (340) reported the outcome of a trial of clenbuterol (30 or 40 μ g/day) for 6 to 18 mo in four human patients with muscular dystrophy, including one patient with Becker type, one with Miyoshi type, and two with facioscapulohumeral dystrophy. According to the abstract from this Japanese study, the researchers found that the most atrophic muscles did not improve with clenbuterol treatment, but in those muscles where mass was better preserved, clenbuterol produced a beneficial effect. They concluded that administration of clenbuterol may be beneficial in the early stage of the different muscular dystrophies (340).

Fowler et al. (140) administered albuterol at a lower dose of 8 mg/day for 28 wk to boys with DMD or Becker muscular dystrophy (BMD) and found modest increases in strength with no side effects. These results suggested that albuterol was well tolerated but elicited only modest improvements in skeletal muscle mass and strength. Thus a more efficacious β -agonist may be required where there is severe wasting and weakness. Based on the data from Harcourt et al. (174) newer generation β -agonists (such as formoterol), which have long-lasting muscle anabolic effects even when administered at very low doses, may provide a better alternative for examining the efficacy of β -agonists for DMD, BMD, or other muscular disorders.

C. Motor Neuron Disease: Amyotrophic Lateral Sclerosis

It has been reported that clenbuterol induces the synthesis of endogenous nerve growth factor (NGF), which may itself be a myotrophic factor released by neuron endings (143, 144, 366). The suggestion that clenbuterol (and other β -agonists) may have neurotrophic effects has led to its investigation for other neuromuscular disorders such as amyotrophic lateral sclerosis (ALS; Lou Gehrig's disease) and related conditions. Using motor neuron degeneration (*mnd*) mice which exhibit lysosomal accumulation of lipofuscin-like material associated with a progressive loss of motor function and strength, Zeman et al. (482) reported that clenbuterol treatment (~ 1 mg \cdot kg $^{-1}\cdot$ day $^{-1}$ in the drinking water) enhanced regeneration of motor neuron axons, opposed the development of motor deficits, and attenuated the decreases in grip strength and muscle mass. In G93A-SOD1 mice, a transgenic murine model of familial ALS, clenbuterol administration (1.5 mg \cdot kg $^{-1}\cdot$ day $^{-1}$ ip) delayed onset of hindlimb weakness, as measured by performance on a rotarod, and slowed disease progression (434). A pilot trial using clenbuterol for ALS has been reported in Italy (417).

D. Denervation and Muscle Unloading

β -Agonist administration has been proposed as a strategy to attenuate the loss of muscle mass (and function) following denervation (6, 7, 19, 137, 213, 241, 279, 281, 480, 483) or muscle unloading (4, 110, 376, 450, 476), with application to the muscle atrophy associated with plaster casting, joint pinning, or extended periods of weightlessness, such as during space flight.

Patients with muscle atrophy following spinal cord injury who were given a β -agonist, metaproterenol (80 mg/day), for 4 wk showed increased forearm muscle size and strength, whereas three patients with spinal cord injury given salbutamol (2 mg/day) for 2 wk showed improvements in the cross-sectional area of their vastus lateralis muscles but no improvement in contractile function (322). In another study, treatment with the β -agonist metaproterenol for 4 wk increased muscle size and strength in patients with muscular atrophy following spinal cord injury (405).

Clenbuterol administration was found to ameliorate denervation-induced atrophy in rat soleus muscles (278). Similarly, Zeman et al. (480) reported that denervated rat soleus, tibialis anterior, and gastrocnemius muscles, but not EDL muscles, contained 95–110% more protein after 2–3 wk of treatment with clenbuterol, than in denervated controls. These changes in muscle protein were supported by similar changes in muscle function and muscle fiber cross-sectional area. The magnitude of the effects of

clenbuterol in sparing the mass and functional capacity of denervated muscles appears greater in slow than in fast muscles (280), but these findings are not entirely consistent across different studies (476). These results indicate that the efficacy of β -agonist administration for the treatment of muscle wasting and weakness may differ, depending on the underlying cause. Regardless, the majority of evidence suggests that β -agonist administration can mimic the effects of normal innervation in (denervated) skeletal muscle and highlights the role of β -adrenergic signaling in the maintenance or remodeling of skeletal muscle.

E. Catabolic Stresses: Burns, Sepsis, Cancer Cachexia, and COPD

There are several conditions where metabolic stress leads to significant muscle wasting and weakness, including cancer cachexia, sepsis, burns, and even COPD (102, 156, 204). For each of these conditions, β -agonists have been proposed as a potential pharmacological intervention to attenuate the ongoing loss of muscle and/or to restore muscle mass.

Cancer cachexia is characterized by weight loss, anorexia, asthenia, and anemia and is inversely correlated with the survival time of the patient, and it always implies a poor prognosis (14, 15). Several studies have shown beneficial effects of clenbuterol treatment on cachexia in rats and mice, particularly with respect to muscle wasting (71, 80, 100, 353, 354), although not all studies have been conclusive. For example, Hyltander et al. (196) reported that clenbuterol treatment did not improve body composition in tumor-bearing adult mice relying on spontaneous food intake, whereas growing animals did benefit from treatment. Administration of the newer generation β -agonist formoterol to tumor-bearing rats and mice reversed the muscle-wasting process that was attributed to both an activation of the rate of protein synthesis and an inhibition of the rate of muscle proteolysis (66). Patients with low skeletal muscle strength and exercise capacity due to chronic heart failure showed no improvement in quadriceps muscle mass, maximal isometric strength, or muscle fatigue following treatment with salbutamol (8 mg twice daily) for 3 wk (176).

It should be acknowledged that the purported repartitioning effects of β -agonists may not be desirable for all catabolic conditions. It is recognized that adipose tissue is no longer considered to be just a fuel reservoir or fat depot but rather as an endocrine organ that releases hormones such as adiponectin and leptin. These so-called adipokines can activate AMP-activated protein kinase that affects skeletal muscle metabolism (329, 439, 444). Thus it is important to consider the implications of β -agonist-induced repartitioning on catabolic conditions such as cancer cachexia since a decrease in

adipose tissue would disturb adipokine signaling and skeletal muscle metabolism. To date, studies have focused on the potential for β -agonists (e.g., formoterol) to exert selective and protective effects on heart and skeletal muscle by antagonizing the protein degradation associated with cachexia (66), effects also thought to be due to a normalization of transcription factors including PPAR γ and PPAR δ in skeletal muscle (147).

In rat models of severe burn injury, clenbuterol treatment increased resting energy expenditure and normalized body mass, muscle mass, and muscle protein content (81). In the same study, treatment of another group of burned rats with nadolol, a β -adrenergic antagonist, exhibited reduced muscle mass and no effect on resting energy expenditure, body mass, or muscle protein content. These results demonstrated that hypermetabolism does not invariably result in loss of lean body mass and suggested that clenbuterol may be useful in preserving muscle mass and protein in catabolic diseases. Similarly, high-dose clenbuterol administration (12 mg/kg, in the diet) increased muscle mass (by $\sim 20\%$), RNA (by $\sim 30\%$), and protein content ($\sim 20\%$) of the gastrocnemius and plantaris muscles of the scalded animals (286). Clenbuterol had no effect on body weight but increased carcass water content. Similar findings were reported by Hollyoak et al. (191) who administered clenbuterol ($2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) subcutaneously via a miniosmotic pump. The findings from these studies indicated quite clearly that β -agonists may be of therapeutic value in inhibiting or reversing muscle atrophy associated with thermal injury (81, 191, 286).

F. Improving Muscle Regeneration and Functional Repair After Injury

Due to their anabolic effects on skeletal muscle, there is considerable potential for β -agonists to promote regeneration of injured skeletal muscles. Evidence comes from the findings of Beitzel et al. (31) and Bricout et al. (57) who found that administration of a β -agonist hastened the functional recovery of regenerating rat skeletal muscles after myotoxic injury with bupivacaine or Notexin, respectively. Daily fenoterol administration to rats ($1.4 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ip) enhanced the force-producing capacity of the injured/regenerating EDL muscles by 19% compared with untreated regenerating muscles at 14 days postinjury. These improvements in contractile function with fenoterol treatment were associated with increases in protein content and fiber cross-sectional area in the regenerating muscles (31). Protein content was higher in regenerating soleus muscles from rats that received daily clenbuterol administration ($2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ by oral gavage) compared with vehicle-treated rats (31). These changes were accompanied by significant slow-to-fast fiber

transitions in the regenerating soleus muscles of clenbuterol-treated rats. β -Adrenergic receptor-mediated mechanisms are thus postulated to play a physiological role in successful muscle regeneration (see sect. IIJ).

In another study, Beitzel et al. (32) studied aspects of β -adrenoceptor signaling during early regeneration of rat EDL and soleus skeletal muscles after bupivacaine injury. Regenerating EDL muscles exhibited a threefold increase in β -adrenoceptor density, which was reduced by 43% following 7 days of fenoterol administration ($1.4 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ip). In the regenerating soleus muscle, β -adrenoceptor density was not altered; however, similar to the EDL, 7 days of formoterol treatment resulted in a 42% reduction in β -adrenoceptor density (32). Despite β -agonist treatment decreasing β -adrenoceptor density in regenerating EDL and soleus muscles, the cAMP response to β -adrenoceptor stimulation, relative to healthy (uninjured) muscles, remained significantly elevated. Thus, despite the marked β -adrenoceptor downregulation within 5 days of β -agonist administration, desensitization is prevented in regenerating muscle by alterations in the G protein population and coupling efficiency, and AC activity, which not only improve signaling and promote the physiological responses required for successful regeneration, but have important implications when considering tissue sensitivity and responsiveness to β -adrenoceptor agonist therapies for promoting muscle repair (32).

A greater understanding of β -adrenergic signaling in skeletal muscle is imperative if this pathway is to be manipulated for the purpose of enhancing muscle fiber growth and functional repair, and for attenuating the loss of muscle fiber size and strength associated with the many conditions that are catabolic to skeletal muscle.

VI. POTENTIAL LIMITATIONS OF β -AGONIST THERAPY AND IMPLICATIONS FOR THEIR ABUSE

Despite the clinical potential of manipulating the β -adrenergic signaling pathway, current approaches stimulating the pathway with β -agonists are not without side effects, and many of these less well-reported deleterious effects have important implications for the health of athletes taking these drugs for performance enhancement. Since the early 1990s, the use of β -agonists for the purpose of enhancing sporting performance has become increasingly prevalent. Despite the so-called desirable effects of increasing muscle bulk and decreasing body fat, many athletes are not aware of the deleterious effects of chronic high-dose β -agonist administration.

The side effects associated with long-term therapeutic use of β -agonists have been detailed previously (2, 61, 220). Excluding athletes, there are two groups of individ-

uals exposed to β -agonists: patients being treated with the drugs and individuals who eat the meat of animals that have been treated with the drugs (23, 148, 312, 390, 420). The most frequently reported side effects associated with the use of β -agonists include nausea, headaches, and insomnia. Excessive use of β -agonists can lead to symptoms such as muscle tremor, palpitations, muscle cramps, headache, and peripheral vasodilatation (361).

Clenbuterol administration has been linked to alterations in animal behavior including increased aggression in mice (290) and suppression of feeding following acute treatment in rats (472). Interestingly, data from Benelli et al. (36) indicated that clenbuterol negatively affects the copulatory behavior of sexually vigorous male rats, but improved that of sexually sluggish ones, providing evidence that central β -adrenoceptor activation can alter behaviors. Clenbuterol has been shown to produce effects on behavior similar to those seen after administration of clinically active antidepressant drugs, indicating that clenbuterol and related β_2 -agonists may have antidepressant activity (323, 332).

Studies on the effects of chronic high-dose β -agonist administration on exercise performance have been conducted on animals. Ingalls et al. (199) subjected mice to a combination of 8 wk of treadmill running (3 sets of 3 min, 36–40 m/min, 10–17% grade, 30-s recovery, 4 days/wk) and clenbuterol treatment (1.6 mg/kg , 4 days/wk) and found that clenbuterol treatment decreased total work performance. Although clenbuterol increased muscle mass, it had antagonistic effects on running performance (199). Clenbuterol administration to rats altered the normal adaptations of skeletal muscle to endurance exercise training (475). Clenbuterol treatment (0.8 mg/kg for 8 wk) decreased glucose transporter (GLUT-4) content within the muscle and decreased citrate synthase activity (242). Other studies have shown that similar high-dose clenbuterol treatments can reduce citrate synthase activity in skeletal muscles (440) as well as decrease capillary density in the left ventricle and skeletal muscles of rats, thereby increasing the diffusion distances for oxygen in the heart and skeletal muscles (427).

A. Effects of β -Agonists on Cardiac Muscle Structure and Function

In addition to these potentially deleterious effects on skeletal muscle, chronic β -agonist administration has been found to have toxic effects on the heart (16, 220). There are three β -adrenoceptors in the heart (214), so it is not surprising that adrenergic stimulation following systemic β -agonist administration can also have major effects on cardiac as well as skeletal muscle (113, 160, 274, 344, 372, 383). Tachycardia (rapid heart beat) is one of the first indications that β -agonists such as clenbuterol are

having an effect. Sudden death due to cardiac failure associated with clenbuterol administration has been reported in both humans (221) and rats during high-intensity swimming exercise (123). Chronic administration of β -agonists such as clenbuterol or salbutamol in rats almost invariably produces significant cardiac hypertrophy (78, 123, 386). Clenbuterol treatment in rats has also been shown to increase cortisol and corticosterone secretions and increase the size of the adrenal glands due to hyperplasia of adrenocortical cells (198).

Cardiac hypertrophy is commonly observed in rats and mice when treated chronically with high doses of β -agonists such as clenbuterol and fenoterol (206, 254, 386, 387, 410). In adult and aged rats treated daily with an intraperitoneal injection of fenoterol (1.4 mg/kg) for 4 wk, cardiac hypertrophy was evident in both groups, and a decrease in cardiac function was observed in the adult rats (160). The cardiac hypertrophy in fenoterol-treated aged rats was associated with an increase in midventricular collagen deposition, whereas adult rats exhibited no change in collagen with treatment. Although previous studies found that β -agonist treatment could increase collagen content in the heart (123), the study by Gregorovic et al. (160) that employed a working heart preparation to evaluate cardiac function suggested that other mechanisms could be responsible for the detrimental effects of high-dose fenoterol treatment in adult rats. Furthermore, areas of apoptotic activity were observed in rat hearts after chronic high dose (5 mg/kg) clenbuterol administration (63), and it is possible that similar damage can contribute to a deterioration of cardiac muscle integrity, collagen infiltration, and impaired cardiac function.

Interestingly, acute periods of clenbuterol administration did not appear to affect cardiac function despite left ventricular hypertrophy (464). 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 (24, 433), although it has been suggested that in rats, the changes to the heart during isoproterenol-induced cardiac hypertrophy are not homogeneous and that myocardial mass, myocardial relaxation, left ventricular stiffness, and systolic function can differ between subgroups of animals (318).

On the contrary, rats administered high doses of clenbuterol (2 mg/kg) daily for several months showed significant cardiac hypertrophy, infiltration of collagen in the left ventricular walls (123), and impaired cardiac mechanics, including reductions in left ventricular pressure (122). Histological examination of the myocardium of dogs following chronic treatment with isoprenaline (in mg/kg doses) revealed severe necrosis (220), while congestion, interstitial edema, hypertrophy of muscle fibers, and myocardial necrosis were evident in rats given very large doses (between 17 and 150 mg/kg daily) of another β -agonist, salbutamol, for 1 mo (256). Severe myocardial

lesions have been observed in the hearts of sheep given intravenous doses of either salbutamol, fenoterol, or isoprenaline (128 μ g/kg at 15-min intervals), for 4 days (341), and isoproterenol treatment produced necrosis and an increase in collagen content in the hearts of rats (42) even when given in low doses (38).

It should also be noted that the β -agonist-induced increases in skeletal and cardiac mass have been utilized favorably in combination with left ventricular devices for treating end-stage cardiac failure to reverse or prevent the adverse effects of unloading-induced cardiac atrophy (347, 416, 446). The rationale is that increasing the frequency and durability of myocardial recovery could reduce or postpone the need for subsequent heart transplantation (470). It is proposed that the cardiac hypertrophy associated with β -agonist administration confers physiological benefits by attenuating myocyte atrophy associated with left ventricular assist devices. Left ventricular device support can restore β -adrenergic receptor signaling in patients with chronic heart failure (231, 345). Birks et al. (43) treated 15 human patients who had undergone implantation of left ventricular assist devices with clenbuterol at an initial dose of 40 μ g twice daily, then at a dose of 40 μ g three times daily, and finally at a dose of 700 μ g three times daily. The dose of clenbuterol was adjusted to maintain the patient's resting heart rate at a level below 100 beats/min. After clenbuterol administration, no serious side effects were observed, but most patients developed a mild tremor, four developed muscle cramps, one developed diaphoresis, and although no new arrhythmias were evident, heart rate increased as expected following β -agonist administration (43). The authors acknowledged that the potential benefits of clenbuterol administration in cases of heart failure should be interpreted with caution, since adverse effects on the heart and the skeletal muscle have been reported in animal models (63, 123, 160).

B. Use of β -Agonists by Athletes for Performance Enhancement

β -Agonist (clenbuterol) usage is highest among bodybuilders for its muscle anabolic properties, but primarily for its lipolytic effects. The exact dosage of clenbuterol that results in the greatest improvements in muscle mass and reductions in body fat has not yet been identified. These criteria are especially important for bodybuilders before competitions where the maintenance of muscle mass is critical during periods of strict dieting. The dosages used by bodybuilders exceed that recommended for asthmatics for therapeutic purposes. Typically, the dose of clenbuterol used ranges from 50–100 or 80–140 μ g/day taken over the course of the day, depending on the individual's tolerance (128). The fact that clenbuterol is not

approved by the United States Food and Drug Administration for use on humans means that little information is available in the scientific literature concerning its use and abuse by athletes (95, 111).

To prevent receptor downregulation (described in sect. 11J), clenbuterol is often used in two or three week "on and off" cycles. Comparing the doses that are effective in rats and translating these for use in humans is obviously difficult due to the differences in size, growth, and metabolism between the species. However, some authors have made interspecies comparisons based on metabolic measurements. For example, Maltin et al. (279) suggested that a dose of 10 $\mu\text{g/kg}$ for the rat was equivalent to 1.0 $\mu\text{g/kg}$ for humans, a dose considered to be safe (121, 122). Even if a theoretical safe dosage of clenbuterol was prescribed for promoting muscle mass in humans, it is unlikely that this level would be adhered to by bodybuilders given that some of these athletes are notorious for taking anabolic steroids in excess of 26 times the therapeutic dose (59, 88, 122). Another confounding issue is the fact that many bodybuilders take more than one drug at any one time, and the supposed increases in muscle mass following clenbuterol administration are hard to gauge when, for example, it is taken in conjunction with either one or more anabolic steroids.

Traditionally, the use of anabolic steroids and growth hormone (GH) has dominated the world of performance-enhancing drugs. However, the use of β -agonists, particularly clenbuterol, for athletic and cosmetic purposes has been increasing steadily (109, 123). The notoriety of clenbuterol emerged during the 1992 Summer Olympic Games in Barcelona, Spain, when two athletes tested positive for its use. Clenbuterol has a long half-life of ~ 35 h (445), and subsequently, the drug will accumulate with repeated doses (323). It can be detected via hair and urine analysis; however, veterinary studies have shown that 97% of the drug will be removed from the body within ~ 10 –11 days (175).

Clenbuterol was banned by the International Olympic Committee on April 21, 1992. Regardless, many athletes still abuse this substance, with most not aware of its potentially lethal side effects when taken in excessive dosages. Sadly, the combination of clenbuterol use with diuretics, for example, has been thought to be responsible for the deaths of several prominent professional bodybuilders (361). There have been case reports describing myocardial infarction in young male body builders either only taking clenbuterol or a combination of clenbuterol and anabolic steroids (153, 221).

It is clear that athletes taking clenbuterol in excessive doses and for extended periods are at greater risk for cardiovascular events. Although many of the side effects (i.e., sweating, tachycardia, and tremor) will cease once the treatment is stopped, the question of whether the deleterious effects on the heart are reversible is more

difficult to answer. Urhausen et al. (447) reported that even several years after discontinuation of anabolic steroid abuse, strength athletes still showed a slight concentric left ventricular hypertrophy compared with drug-free strength athletes. Evidence from animals treated chronically with a high dose of clenbuterol indicates that the deposition of noncontractile fibrotic material in the ventricular walls is likely to affect cardiac mechanics and impair exercise performance. Based on its deleterious and potentially lethal side effects, athletes would be advised not to experiment with these compounds for non-medical use (10).

VII. CONCLUDING REMARKS

This review has provided evidence for the importance of β -adrenergic signaling in skeletal muscle. Although we are only beginning to understand the significance of the β -adrenergic signaling pathway in skeletal muscle, especially in relation to its role in health and disease, a wealth of information exists regarding the stimulation of the β -adrenergic system with β -agonists. The action of β -agonists on β -adrenoceptors in smooth muscle facilitates their life-saving role in the prevention and treatment of bronchospasm in asthma. On the other hand, most evidence obtained from rigorously controlled animal studies has found that chronic stimulation of the β -adrenoceptors in skeletal muscle can elicit anabolic effects. This knowledge has served as the basis for many of the potential therapeutic applications of β -agonists for skeletal muscle wasting disorders, including many neuromuscular diseases, aging, and several metabolic conditions that cause muscle catabolism. Although there is great promise that β -agonists can be used for treating these conditions, their clinical application has been limited by cardiovascular side effects, especially when β -agonists are administered chronically and at high doses. Newer generation β -agonists (such as formoterol) have been shown to elicit an anabolic response in skeletal muscle even at very low doses, and this has renewed enthusiasm for their clinical application, especially because they exhibit 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, so it is important to refine their development. In so doing, it is hoped that beneficial effects of β -agonists can find application to these severe muscle-wasting conditions that impact not only on the ability to perform the tasks of daily living, or quality of life, but ultimately on life itself, since the maintenance of functional muscle mass is critical for survival. It is likely that a greater understanding of the β -adrenergic signaling pathway in skeletal muscle will

reveal novel targets that will facilitate the development of new therapeutic strategies, ones that manipulate pathways that benefit skeletal muscle by increasing protein synthesis or reducing protein degradation, without simultaneously activating pathways that affect the cardiovascular system deleteriously.

Despite warnings to athletes about the potential side effects of using β -agonists for athletic performance or enhancing physical appearance, there is no doubt that these drugs will continue to be used either alone or in conjunction with other compounds. It is hoped that the wealth of evidence presented in this review regarding the effects of β -agonists on skeletal muscle (and on other tissues) might help to encourage athletes not using these drugs safely for the purpose they were originally intended (i.e., bronchospasm), to reconsider their actions. Although very promising, the therapeutic potential of β -agonists for muscle-wasting conditions will not be realized until all aspects relating to their safety can be established, especially for their chronic, long-term administration necessary for attenuating the loss of muscle size and strength in these severe muscle-wasting conditions.

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